Chromosomes of Archaeogastropoda (Mollusca : Prosobranchia), with Some Remarks on Their Cytotaxonomy and Phylogeny

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Chromosomes of Archaeogastropoda (Mollusca: Prosobranchia), with Some Remarks on Their Cytotaxonomy and Phylogeny

By

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With Text-figures 1–35 and Tables 1–18

Abstract Karyotype of 27 species of eight archaeogastropod families were investigated. Including these results, karyological information on the Archaeogastropoda was reviewed based on the data stored in CISMOCH (Computerized Index System for Molluscan Chromosomes).

Conservation in chromosome number and morphology has been urged and was confirmed in major archaeogastropod groups: Patellacea (Acmaeidae and Patellidae), Neritacea (marine neritids) and Trochacea (Trochidae, Turbinidae and Stomatellidae). These archaeogastropods have certain characteristic karyotypes including chromosome number, size and morphology. Remarkable diversification in karyotype has occurred primarily in special groups having a secondary limpet-form or expanding from the sea to brackish/fresh-water areas.

No results have been obtained in support of the hypotheses on an evolutionary tendency of change in chromosome number, while evolutionary polyploidy is hypothesized to have occurred in a hermaphroditic ancestor at a very early stage of gastropod radiation.

Introduction

The first studies in the field of molluscan cytology date from the late nineteenth century. Because of inferior optical equipments and method, however, many of the early reports have been shown to be inaccurate (Patterson & Burch, 1978). In recent years, especially since 1960, a considerable amount of information has been accumulated on variable taxa of Mollusca. This recent interest may be attributed to the boom in the field of cytogenetics, in particular that of mammalian cytogenetics. In Harvey's list (1920) chromosome numbers of only 44 molluscan species were recorded, and in Makino's list (1950) those of only 127 species and subspecies. Then just two decades after Makino's work, the chromosome numbers of 622 species and subspecies were reported from 1930 to 1969 (Patterson, 1969). Chromosome numbers are now known for members of five molluscan classes, i.e., Gastropoda, Bivalvia, Polyplacophora, Cephalopoda and Scaphopoda. Most of the results have been reported from gastropods, in which only the two pulmonate orders, Basommatophola and Stylommatophola, are at all well surveyed.

Though Archaeogastropoda, a prosobranch order, is thought to be the stem of the gastropod phylogenetic tree, and other orders are conceived as more advanced and probably evolved from this primitive order (Cox, 1960; Salvini-Plawen, 1980;

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and others), karyological information on this group is comparatively scarce: chromosome numbers of only 32 species were listed in Patterson's review (Patterson, 1969). These early works provide only fragmentary information since they generally aimed at the establishment of chromosome counts, often using meiotic preparations with sectioning method, and therefore the chromosomes were poorly characterized. Under these circumstances I have been studying the animals of the Archaeogastropoda, and have reported the chromosomes of 13 species in five families since 1982.

The objectives of my earlier studies were (1) to determine the chromosome number and describe the karyotype in detail because chromosome number alone shed little light on the cytotaxonomical relationships, and (2) to recheck previous counts of the chromosomes and descriptions of their gross morphology. I have been developing a technical method for the chromosome preparation and measurement to achieve these objectives. Continuing the course of my study, I have several additional aims to present this paper: first, I will discuss the technical problems, and present an easy and applicable method to raise the level of chromosome analysis in the molluscan field; and second, I will report further information on the chromosomes of 27 species from eight families of major archaeogastropod superfamilies to fill in the deficit in the studies of this field.

My third aim of this paper is to present a list from CISMOCH, Computerized Index System for Molluscan Chromosomes, of the information so far reported on the Archaeogastropoda chromosomes. More than fifteen years have passed since the Patterson's review was published. During this period chromosomal information on various species of molluscs has continuously been accumulating. In order to make full use of this enormously growing information, I have been developing a computerized reference system, CISMOCH, which is a simplified version of the system developed by Ojima and his students in the field of fish cytotaxonomy (Fujii & Ojima, 1983; Ojima, 1984). Finally, I will review karyological characters, remark on the cytotaxonomy of the Archaeogastropoda, and present some systematic interpretations with discussing the karyological tendencies among the molluscs.

Observation on the Chromosomes of 27 Archaeogastropod Species

Materials and methods

Twenty-seven species from eight families in the Archaeogastropoda were karyologically investigated. Most of the specimens were collected from the sea-shore near the Seto Marine Biological Laboratory, on the southwest coast of Kii Peninsula, Honshu, Japan. Table 1 shows the localities and dates of collection, and the number and sex of examined snails and cells.

For the chromosome study the materials were prepared by the warm-dry method of Kligerman & Bloom (1977) with some modifications:

1) The snails were kept in 0.005–0.01% colchicine solution for 6–12 hours before being sacrificed.
2) Removed gonad was cut into small pieces and soaked in 0.075 M KCl hypotonic solution.
3) These pieces were fixed in freshly mixed Carnoy's fixative (3:1 methyl alcohol acetic acid).
4) Tissues were then minced gently in 50% acetic acid to prepare a cell suspension.
5) A drop of the cell suspension was pipetted by a microhematocrit capillary tube (ϕ 0.85 mm)

Table 1. Localities and dates of specimen collection, and number of examined snails and cells.

Species	Locality	Date	Examined snails	Cells for counting chromosome no. ⟨N⟩	⟨2N⟩	Measured chromosome sets
Acmaeidae						
Patelloida striata	Toka, Okinawa	Jan. '81	m2	m4	m3	m1
P. saccharina lanx	Shirahama, Wakayama	July '84	m7	m14	m29	m11
Patellidae						
Cellana grata	Shirahama, Wakayama	July '81, '83	m7	m7	m35	m13
C. nigrolineata	Shirahama, Wakayama	July '84	m3	m6	m8	m5
Patella flexuosa	Shirahama, Wakayama	July '82, '83	m8	m10	m25	m6
Neritidae						
Nerita albicila	Shirahama, Wakayama	May, June '83, '84	f5, m9	f6, m57	f8, m17	f4, m3
N. helicinoides laevilabris(1)	Gizabanta, Okinawa	Mar. '82	m2	m8		
N. insculpta(1)	Gizabanta, Okinawa	Mar. '82	m2	m2	m2	
N. japonica	Shirahama, Wakayama	Apr., May '84	f5, m4	m10	f22, m6	f1, m3
N. plicata(1)	Gizabanta, Okinawa	Mar. '82	m2		m6	
Haliotidae						
Haliotis gigantea(2)	Tateyama, Chiba	Sept. '84	m1	m7		
H. varia	Shirahama, Wakayama	Sept. '84	m7	m13	m14	m5
Fissurellidae						
Diodora quadriradiatus	Hoi Sing Wan, H.K.	Apr. '83	m2		m52	m6
Tugali decussata(3)	Shirahama, Wakayama	Apr. '85	f1, m2	f5, m6	f8	f1
Macroschisma dilatata	Shirahama, Wakayama	May '84	m5	m6	m28	m3
Montfortula pulchra picta	Shirahama, Wakayama	June '82, '83	m7	m14	m47	m4
Trochidae						
Monodonta australis(3)	Chichijima, Bonin Is.	June '80	m1		m10	
M. labio confusa	Shirahama, Wakayama	May '81, '84	m8	m9	m18	m7
M. neritoides	Shirahama, Wakayama	Apr. '81	m6	m5	m15	m3
M. perplexa	Shirahama, Wakayama	May '81	m5	m11	m10	
Pictodiloma suavis	Shirahama, Wakayama	July '82	m5	m6	m8	
Chlorostoma argyrostoma lischkei	Shirahama, Wakayama	May '82, '84	m6	m9	m7	
C. nigricolor	Shirahama, Wakayama	May '82	m3	m5	m4	
Omphalius nigerrima	Shirahama, Wakayama	Apr. '81	m3	m7	m6	m1
Granata lyrata(3)	Sakurajima, Kagoshima	May '84	m2	m3	m2	
Turbinidae						
Lunella cornata coreensis	Shirahama, Wakayama	June '81	m3	m4	m10	m5
Stomatellidae						
Broderipia iridescens(3)	Shirahama, Wakayama	Apr. '85	m2	m8	m7	m1

Specimens were provided by (1) Kurozumi, (2) Koike and Yamakawa, and (3) Takenouchi.
f: female m: male

and placed onto a heated clean glass slide (about 50°C).

6) The suspension drop was immediately withdrawn back into the capillary tube so as to leave the cells on the periphery of the drop in a ring on the slide.
7) Steps 5 and 6 were repeated to produce two or three rings on a slide.
8) The cells left on the slide were dried, and then stained for 20 minutes in a 2% Giemsa (Merk) solution made up in 0.1 M phosphate buffer at pH 6.8.
9) The stained slide was rinsed briefly and dried by applying warm air from a blower, and then placed in two changes of xylene for several minutes and mounted in Canada balsam.

The prepared slides were observed under a Nikon Optiphot microscope with a 100× (n.a. 1.25) oil immersion objective and a 10× ocular. Photographs were taken with a Nikon Microflex UFX system on Fuji Minicopy MR2 (ASA 12) high contrast film and enlarged on Fujibro WP paper, either F4 or F3, to give a final magnification usually about 3200×.

The metaphase spreads were submitted to computer-assisted analysis using a system similar to that described as CROMPAC by Green et al. (1980). Ordering of chromosomes by size, pairing of homologues, calculation of relative lengths and arm ratios, and categorization were accomplished or facilitated by the computer. The system was designed and presented in use with a Sord M-100 ACE 8-bit microcomputer with 64K bytes of random access memory (RAM). Memory storage is on 5-inch floppy magnetic discs.

Digitizing of chromosome measurements was done at high magnification (5000×) with a Mitutoyo digitizer which was mounted on a Nikon Shadowgraph contour projection. Negatives of chromosome spreads were displayed on the screen of the projection and measured.

The following morphological features were used to compare the karyotypes: (1) Relative length (R.L.) of the chromosomes, percentage of the total length of the autosomes. (2) Arm ratio (A.R.) obtained by dividing the length of the short arm into that of the long arm of the chromosomes. Nomenclature of chromosome types was adopted according to the "non-recommended" terminology of Levan et al. (1964): metacentric, M (A.R. 1.00–1.69); submetacentric, SM (1.70–2.99); subtelocentric, ST (3.00–6.99); and telocentric, T (7.00 and above).

The measurements were performed mostly according to Bentzer's indication (Bentzer et al., 1971) on the metaphase figures selected at an identical constriction level, as deep condensation makes it difficult to observe primary constriction and other karyological characteristics.

Remarks on the preparation technique.

The majority of the reports on molluscan chromosomes mainly deals with the determination of chromosome number, mostly in the meiotic plates. Only a few investigators have succeeded in the more detailed analysis on the karyotypes. This state of our information on molluscan chromosomes has been said to be mainly due to the small size of the chromosomes and to technical difficulties (Ramammorthy, 1958; Burch, 1968). It is generally admitted, however, that the chromosome number alone can shed little light on the phylogenetic relationships of organisms. Therefore, the technique that can overcome the difficulties in producing more informative karyological features is needed. The preparation method that I have developed presents a good quality metaphase to show the location of the centromere as well as chromosome numbers, making morphological analysis of the chromosome arms possible. Moreover, by using this method, such high quality metaphase can be found easily because it is located on the periphery of the cell ring. On the contrary, when using common flame-dry and air-dry methods, finding metaphase spreads in the prepared slide is rather difficult.

Supposedly, chromosomes are obtainable from any eukaryotic organs whose cells are actively dividing. There must be a variety of tissues in molluscs that may be

Table 2. Chromosome numbers of the Archaeogastropoda examined in the present study.

Species	Chromosome Number 〈N〉	〈2N〉
Acmaeidae		
Patelloida striata	m10	m20
P. saccharina lanx	m10	m20
Patellidae		
Cellana grata	m9	m18
C. nigrolineata	m9	m18
Patella flexuosa	m9	m18
Neritidae		
Nerita albicila	f12	f24 (22+xx)
	m12 (11+h)	m23 (22+x)
N. helicinoides laevilabris	m12	
N. insculpta		m23 (?22+x)
N. japonica	f12	f24 (22+xx)
	m12 (11+h)	m23 (22+x)
N. plicata	m12 (11+h)	m23 (?22+x)
Haliotidae		
Haliotis gigantea	m18	
H. varia	m16	m32
Fissurellidae		
Diodora quadriradiatus		m32
Tugali decussata	f, m15	f30
Macroschisma dilatata	m16	m32
Montfortula pulchra picta	m14	m28
Trochidae		
Monodonta australis		m36
M. labio confusa	m18	m36
M. neritoides	m18	m36
M. perplexa	m18	m36
Pictodiloma suavis	m18	m36
Chlorostoma argyrostoma lischkei	m18	m36
C. nigricolor	m18	m36
Omphalius nigerrima	m18	m36
Granata lyrata	m20?	m40?
Turbinidae		
Lunella cornata coreensis	m18	m36
Stomatellidae		
Broderipia iridescens	m18	m36

f: female m: male h: heterochromatic chromosome x: sex chromosome

selected for obtaining chromosomes: mantle, kidney, embryos and so on. As I previously pointed out (Nakamura, 1985a), recent workers tend to concentrate on the karyotype analysis of the mitotic metaphase chromosomes and as a consequence meiotic observations tend to be neglected. But it is in the meiosis that sex and other special chromosomes reveal their characters (Vorontsov, 1973). Therefore the chromosome in both meiotic and mitotic stages should be observed equally. In this respect gametogenetic gonads, especially male testes, are probably the best because they yield both configurations simultaneously. The method adopted in the present work, originally elaborated for obtaining chromosomes from solid tissues of fishes, is also very applicable for the 'soft' tissue of molluscan testis.

The advantage of the squash technique, used most frequently in the field of molluscan karyology, is that it is quick, while the major drawback with it is that once applied, it cannot be stopped. In the procedure of the present warm-dry method, there are some steps which allow a break. After step 4, tissues can be stored in fixative for a few months below 4°C without apparent deterioration. If the fixative is occasionally renewed, tissues can be preserved much longer, even for several years. After step 7, the preparations can also be stored in refrigerator in a cover container for several months before staining. Only in case that banding stain techniques are to be applied, the procedures should be finished without any breaks to obtain constant results.

Results

The species used for the present investigation and their chromosome numbers are summarized in Table 2.

Acmaeidae (Patellacea)

1. *Patelloida striata* Quoy et Gaimard, 1834

Two males were cytologically examined. Chromosome numbers of 2N=20 and N=10 were counted (Fig. 1). The karyotype of the spermatogonial metaphase chromosomes consisted of 6 pairs of the large M and 4 pairs of the smaller chromosomes. Two of the smaller pairs were ST and SM chromosomes. The smallest two pairs were probably M for No. 9 and T for No. 10 pair. Only one set of the chromosomes could be measured and the results are shown in Table 3. Chromosome length ranged from 1.03 to 3.60 μm and the total length was 50.89 μm. In the meiotic plates each bivalent seemed to be equally stained and no special behaviour of chromosomes was observed.

2. *Patelloida saccharina lanx* (Reeve, 1855)

Meiosis in males was observed. At zygotene/pachytene the chromosomes were arranged in a bouquet and have many tiny, well-stained chromatin dots (Fig. 2-A). At diakinesis and metaphase I, 10 bivalents could be counted in all figures (Fig. 2-A & B) and were stained equally. At diakinesis they appeared as rings and dots.

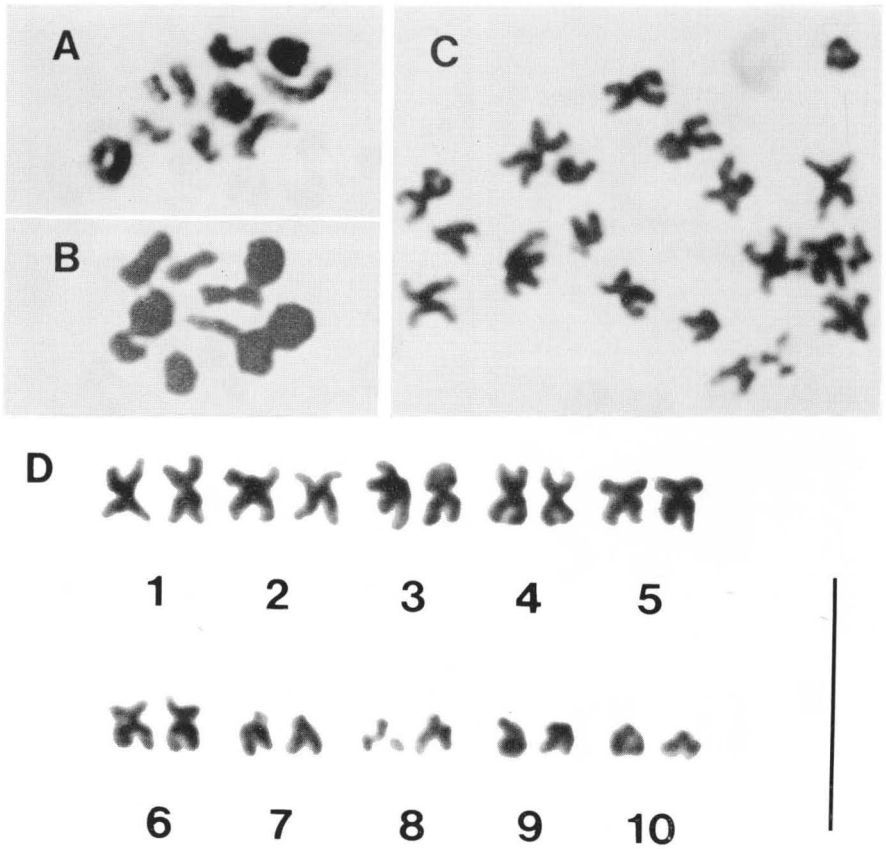

Fig. 1. Chromosomes of male *Patelloida striata*. A, B. Early metaphase I (N=10); C. Spermatogonial metaphase (2N=20); D. Karyotype constructed from Fig. 1-C. Scale bar: 10 μm.

Table 3. Measurements of chromosomes at the mitotic metaphase of *Patelloida striata* and *P. saccharina lanx*.

Chromosome	*Patelloida striata*			*P. saccharina lanx*		
	RL	AR	Type	RL±SE	AR±SE	Type
1	13.96	1.09	M	12.88±0.17	1.22±0.03	M
2	12.99	1.07	M	11.85±0.08	1.23±0.03	M
3	12.80	1.06	M	11.22±0.10	1.20±0.04	M
4	12.07	1.27	M	10.90±0.10	1.51±0.05	M
5	11.24	1.38	M	10.21±0.11	1.16±0.03	M
6	10.91	1.50	M	9.62±0.06	1.27±0.05	M
7	7.82	3.17	ST	9.05±0.09	1.22±0.04	M
8	7.59	2.02	SM	8.74±0.11	1.48±0.04	M
9	5.73	1.37	M	8.11±0.10	1.22±0.06	M
10	4.54	—	T	7.30±0.11	1.32±0.05	M
TCL	50.89			52.84±1.22		

The chromosome pairs of the karyotype are arranged by size. RL: relative length of the chromosomes, percentage of the total length of the autosomes in haploid. AR: arm ratio, obtained by dividing the length of the short arm into that of the long arm of the chromosome. Type: nomenclature after Levan, et al. (1964). TCL: total length of the autosomes in diploid. SE: standard error.

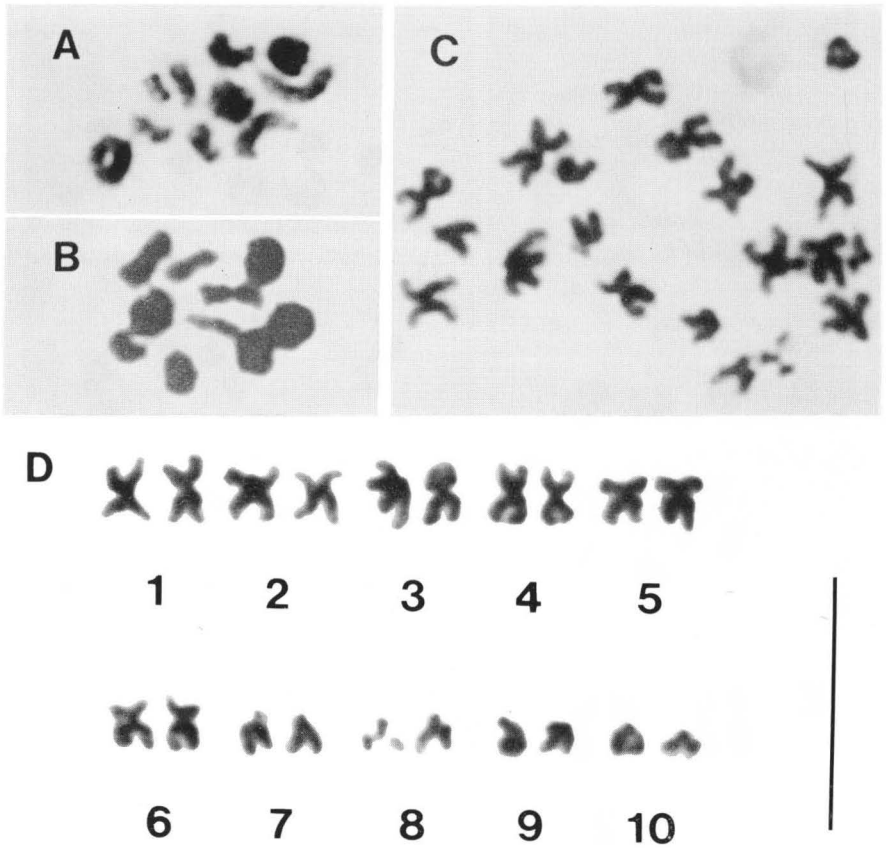

Fig. 1. Chromosomes of male *Patelloida striata*. A, B. Early metaphase I (N=10); C. Spermatogonial metaphase (2N=20); D. Karyotype constructed from Fig. 1-C. Scale bar: 10 μm.

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	RL	AR	Type	RL±SE	AR±SE	Type
1	13.96	1.09	M	12.88±0.17	1.22±0.03	M
2	12.99	1.07	M	11.85±0.08	1.23±0.03	M
3	12.80	1.06	M	11.22±0.10	1.20±0.04	M
4	12.07	1.27	M	10.90±0.10	1.51±0.05	M
5	11.24	1.38	M	10.21±0.11	1.16±0.03	M
6	10.91	1.50	M	9.62±0.06	1.27±0.05	M
7	7.82	3.17	ST	9.05±0.09	1.22±0.04	M
8	7.59	2.02	SM	8.74±0.11	1.48±0.04	M
9	5.73	1.37	M	8.11±0.10	1.22±0.06	M
10	4.54	—	T	7.30±0.11	1.32±0.05	M
TCL	50.89			52.84±1.22		

The chromosome pairs of the karyotype are arranged by size. RL: relative length of the chromosomes, percentage of the total length of the autosomes in haploid. AR: arm ratio, obtained by dividing the length of the short arm into that of the long arm of the chromosome. Type: nomenclature after Levan, et al. (1964). TCL: total length of the autosomes in diploid. SE: standard error.

others), and this may be the reason for the difference. Moreover, Colombera (pers. comm.) counted the same chromosome number of N=10 in this species as the present result. Therefore it must be concluded that this species has the chromosome number of N=10.

Twenty chromosomes were observed at spermatogonial metaphase (Fig. 2-D). Fig. 2-E shows the karyotype arranged by size and Table 3 shows chromosome measurements of this species. There were no sharp distinctions, and the chromosomes gradually decreased in size from No. 1 pair to No. 10 pair. All of the chromosomes were M; observed minimum length of No. 10 chromosome was 1.64 μm and the maximum of No. 1 was 4.08 μm. The mean total length of the chromosomes in the diploid was 52.84$\pm$1.22 μm.

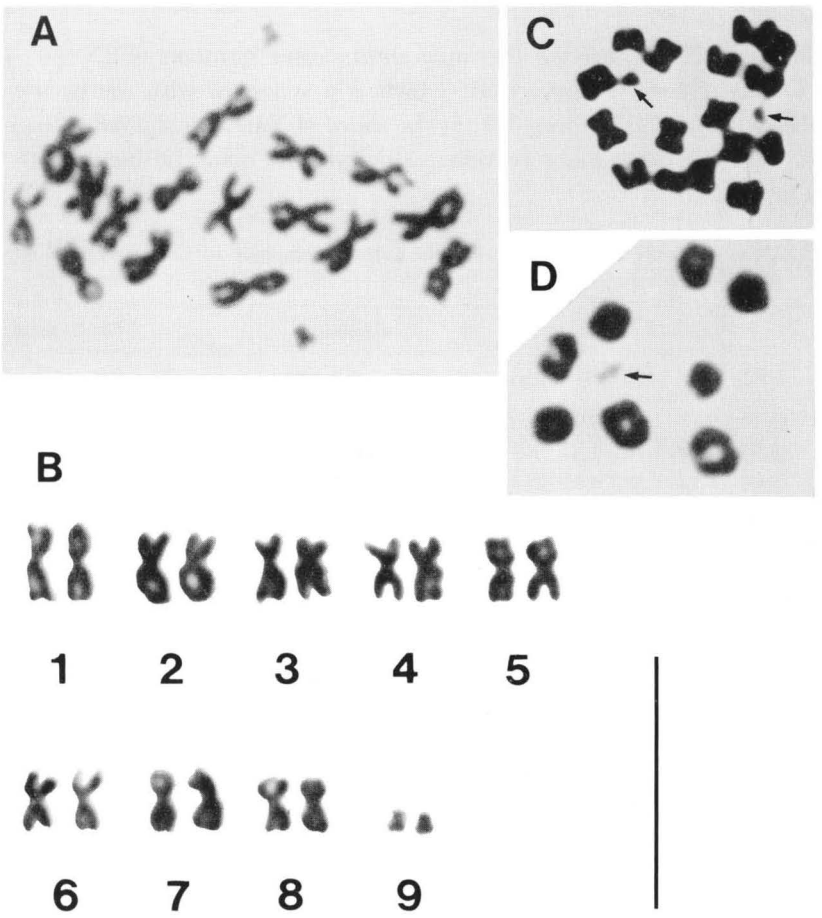

Fig. 3. Chromosomes of male *Cellana grata*. A–C: Mitosis. D: Meiosis. A. Spermatogonial metaphase (2N=18); B. Karyotype constructed from Fig. 3-A; C. Deeply condensed chromosomes at spermatogonial metaphase (Arrows indicate the smallest chromosome pair); D. Metaphase I (N=9) (An arrow indicates the smallest bivalent). Scale bar: 10 μm.

Patellidae (Patellacea)

3. *Cellana grata* (Gould, 1859)

Eighteen chromosomes were counted at spermatogonial metaphase (Fig. 3-A). Fig. 3-B is the karyotype constructed from the chromosomes shown in Fig. 3-A. All chromosomes were M in which the smallest No. 9 pair was clearly distinguishable from the others by its lightly stained color, as well as its remarkably small size, regardless of the degree of chromosome condensation (Fig. 3-C). Chromosome measurements are shown in Table 4. Observed chromosomes ranged from 1.02 to 3.75 μm in length. The mean$\pm$its standard error of the total metaphase chromosome length in mitosis was 47.73 ± 0.77 μm.

At the first meiotic metaphase in males nine bivalents were observed and the smallest bivalents could be clearly distinguished as it was stained more weakly than the others (Fig. 3-D).

Nishikawa (1962) reported the same chromosome numbers of 2N=18 and N=9 from *Cellana eucosmia* Pilsbry, 1891, which is a synonym with the present species (see Habe & Ito, 1974), collected from the shore of Yamaguchi Pref. His counting coincides with the present observation. However, he made no mention of the No. 9 chromosome.

Table 4. Measurements of chromosomes at the mitotic metaphase of *Cellana grata*, *C. nigrolineata* and *Patella flexuosa*.

Chromosome	*Cellana grata*			*C. nigrolineata*			*Patella flexuosa*		
	RL$\pm$SE	AR$\pm$SE	Type	RL$\pm$SE	AR$\pm$SE	Type	RL$\pm$SE	AR$\pm$SE	Type
1	14.12$\pm$0.12	1.21$\pm$0.03	M	14.46$\pm$0.16	1.23$\pm$0.05	M	14.42$\pm$0.17	1.28$\pm$0.06	M
2	12.95$\pm$0.10	1.19$\pm$0.03	M	13.10$\pm$0.12	1.30$\pm$0.09	M	13.67$\pm$0.25	1.22$\pm$0.06	M
3	12.67$\pm$0.13	1.50$\pm$0.06	M	12.14$\pm$0.13	1.42$\pm$0.11	M	12.76$\pm$0.16	1.16$\pm$0.03	M
4	12.03$\pm$0.03	1.27$\pm$0.04	M	11.43$\pm$0.05	1.19$\pm$0.05	M	12.22$\pm$0.15	1.27$\pm$0.06	M
5	11.54$\pm$0.08	1.14$\pm$0.02	M	11.10$\pm$0.12	1.49$\pm$0.10	M	11.12$\pm$0.21	1.21$\pm$0.07	M
6	10.91$\pm$0.13	1.15$\pm$0.02	M	10.69$\pm$0.16	1.40$\pm$0.09	M	10.62$\pm$0.15	1.19$\pm$0.04	M
7	10.41$\pm$0.18	1.40$\pm$0.04	M	10.61$\pm$0.11	1.17$\pm$0.04	M	10.19$\pm$0.12	1.25$\pm$0.07	M
8	9.50$\pm$0.14	1.16$\pm$0.03	M	9.58$\pm$0.20	1.31$\pm$0.07	M	9.60$\pm$0.22	1.16$\pm$0.04	M
9	5.79$\pm$0.18	1.47$\pm$0.06	M	6.81$\pm$0.25	1.39$\pm$0.12	M	5.31$\pm$0.22	—	T
TCL	47.73$\pm$0.77			37.38$\pm$1.53			57.41$\pm$4.49		

See Table 3 for explanations.

4. *Cellana nigrolineata* (Reeve, 1854)

Eighteen chromosomes were observed at spermatogonial metaphase (Fig. 4-A). Though the chromosomes seemed to be slightly over-condensed, probably because of over-treatment with colchicine, the smallest No. 9 chromosome pair could be clearly distinguished from the others because of its light color as well as its size. All the chromosomes were metacentrically contructed (Fig. 4-B). Chromosome measurements are shown in Table 4. Observed chromosomes ranged from 1.06 to 2.77 μm

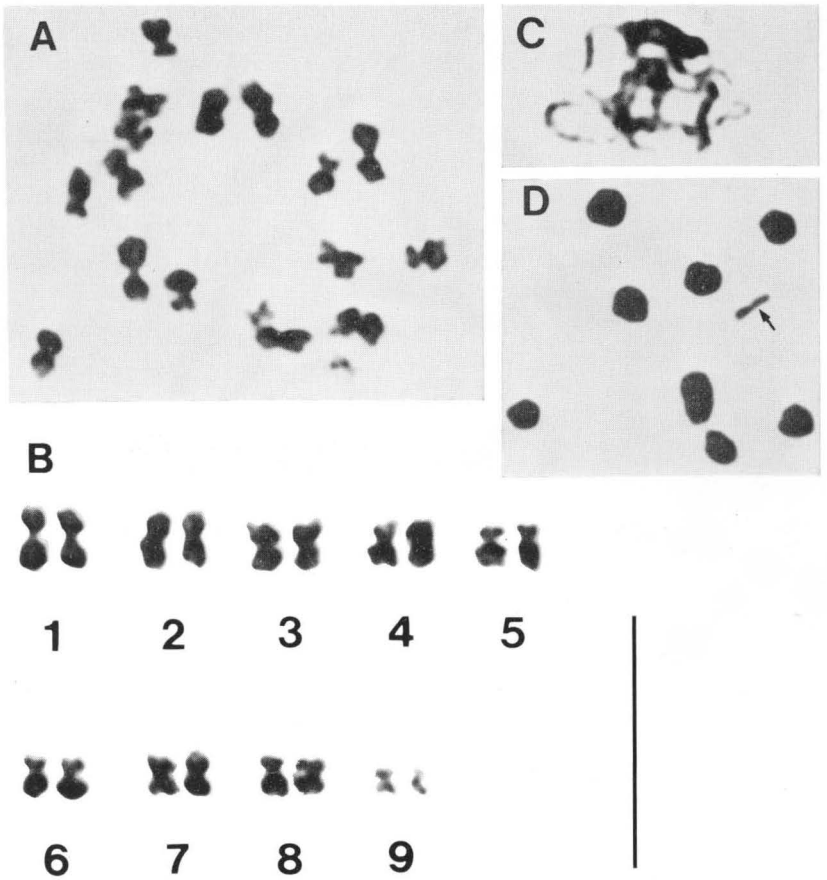

Fig. 4. Chromosomes of male *Cellana nigrolineata*. A–B: Mitosis. C–D: Meiosis. A. Spermatogonial metaphase (2N=18); B. Karyotype constructed from Fig. 4-A; C. Prophase I (zygotene); D. Metaphase I (N=9) (An arrow indicates the smallest bivalent). Scale bar: 10 μm.

in length. Mean total length of mitotic chromosomes was $37.38 \pm 1.53\ \mu$m.

The chromosomes in male meiosis were also observed. At prophase I all the chromosomes were long threads in a bouquet arrangement and no heterochromatic chromosomes (h-chromosomes) were observed (Fig. 4-C). Nine bivalents including a small, lightly-stained one could be counted at metaphase I (Fig. 4-D). Nishikawa (1962) reported a haploid chromosome number of nine from this species, which coincided with the present observation. He made no mention about the smallest chromosomes, nor do his camera lucida drawings show it, which may be attributed to his technique, as mentioned previously.

5. *Patella flexuosa* Quoy et Gaimard, 1834

The chromosomes in male were observed. Many cells at prophase I were found, when homologues were getting shorter and thicker but each still resembled a single, two-stranded bivalents (Fig. 5-A). No h-chromosomes were observed

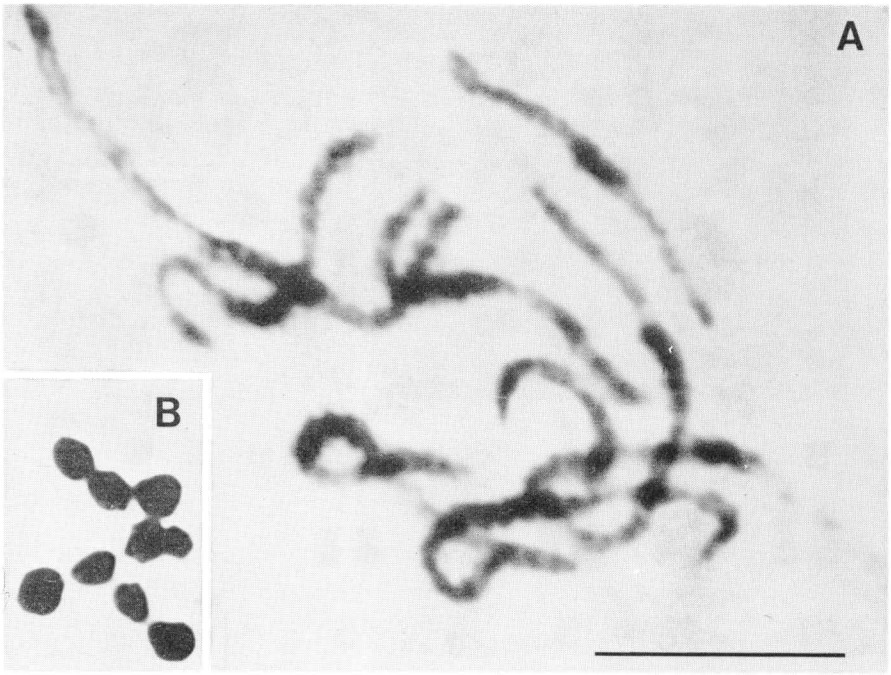

Fig. 5. Meiosis in male *Patella flexuosa*. A. Prophase I (pachytene); B. Metaphase I (N =9). Scale bar: 10 μm.

during prophase stages. Nine bivalents could be counted at metaphase I (Fig. 5-B). In this stage all the bivalents were usually stained equally and they were not always easily distinguishable from each other only by size.

Eighteen chromosomes were observed at spermatogonial metaphase (Fig. 6-A & B). As colchicine, the spindle inhibitor, probably did not work efficiently, the condensation of the chromosomes was variable in each cell. Mean total chromosome length was $57.41 \pm 4.49\ \mu$m and observed chromosomes ranged from 1.22 to 5.66 μm in length. Fig. 6-C is the karyotype constructed from the chromosomes shown in Fig. 6-B, which was one of the most elongated complements. The larger eight pairs were M chromosomes with a gradual size gradient. The smallest No. 9 pair was clearly distinguished from the others by its shape (lacking short arms, i.e., the T chromosome), as well as by its small size. This No. 9 pair was stained deeply just as the others. Chromosome measurements are shown in Table 4.

This is the second report on the chromosomes of the genus *Patella*. Vitturi et al. (1982) reported the same chromosome numbers of N=9 and 2N=18 from *P. vulgata* Linaeus, 1758, the commonest limpet in Europe. However, its karyotype is different from that of the present species: *P. vulgata* has two more T chromosome pairs.

Neritidae (Neritacea)

6. *Nerita albicila* Linnaeus, 1758

The chromosomes in male and female meiosis were observed. In early male

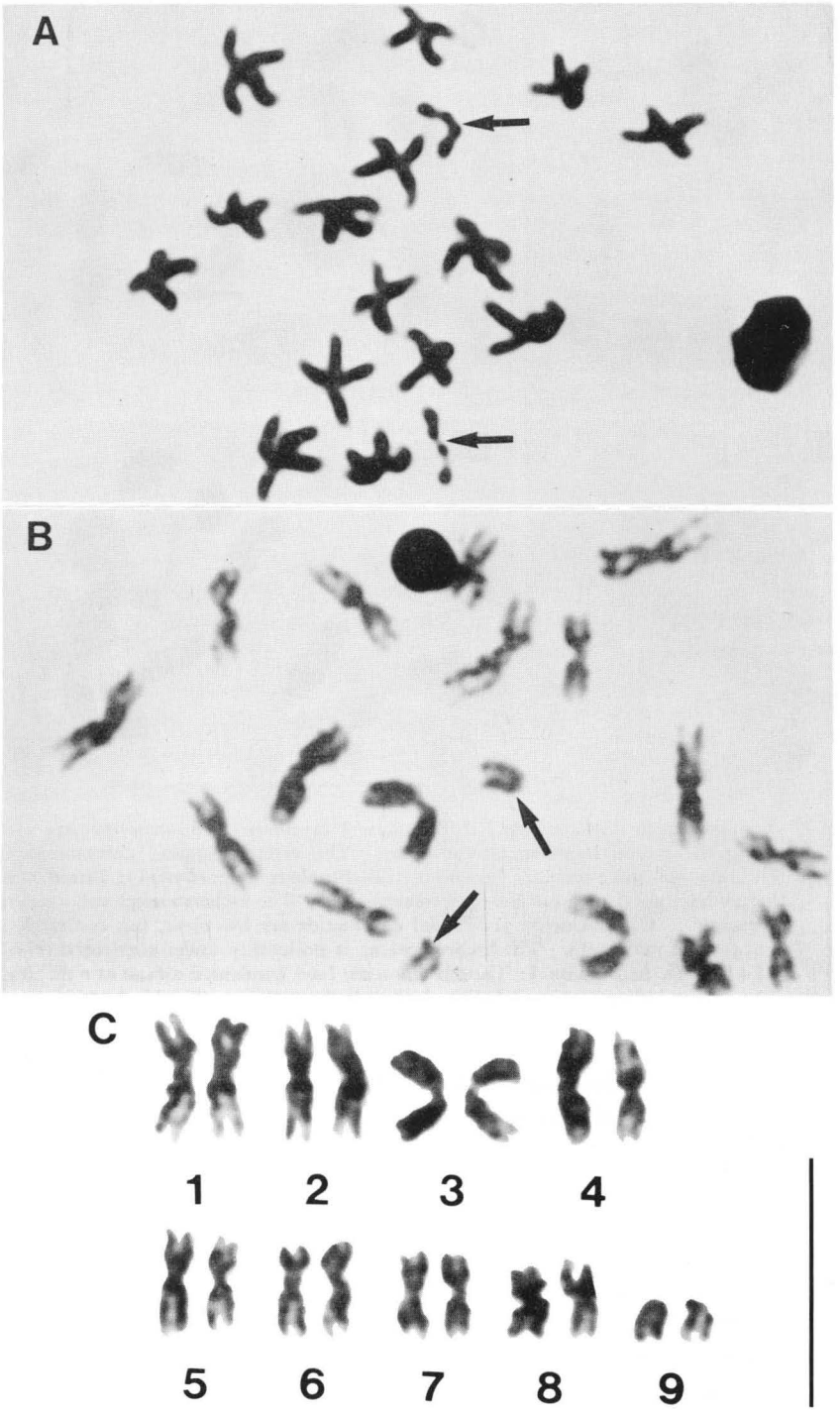

Fig. 6. Chromosomes of *Patella flexuosa*. A, B. Spermatogonial metaphase (2N=18) (Arrows indicate the smallest T pair). C. Karyotype constructed from Fig. 6-B. Scale bar: 10 μm.

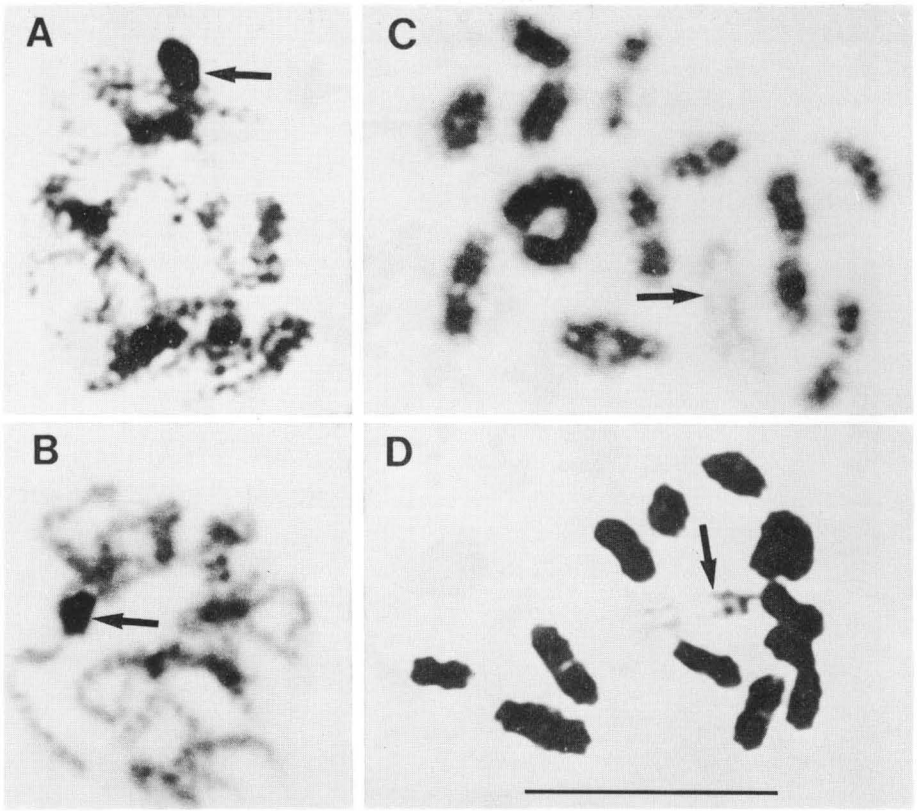

Fig. 7. Meiosis in male *Nerita albicila*. A. Prophase I (zygotene): Chromosomes are visible along the paired homologous autosomes. The heterochromatic chromosome (h-chromosome) still condenses (arrowed). B. Prophase I (pachytene): Paired homologues have condensed further to become shorter. The h-chromosome still condenses (arrowed). C. Diakinesis: Individual chromatids are less clear, but contraction is not yet at a maximum. The h-chromosome is noticeably under-condensed (N=12, 11+h). D. Metaphase I: The chromosome have condensed almost at a maximum (An arrow indicates an under-condensed h-chromosome (N=12, 11+h)). Scale bar: 10 μm.

meiotic prophase I, the chromosomes formed a weakly-stained, net-like structure, in which many, minute, well-stained chromation dots could be seen (Fig. 7-A & B). An h-chromosome was distinguishable as a condensed mass, the so-called heteropycnotic chromosome. Next the h-chromosome became rather straightened and was then distinguishable as a less contracted complement, the so-called negative heteropycnotic chromosome, at diakinesis and metaphase I (Fig. 7-C & D). The h-chromosome at metaphase I was sometimes observed to have a small contraction in the chromatid, resembling a thread with a node. Twelve chromosomes including one h-chromosome could be counted at these stages. Komatsu & Inaba (1982) reported the same chromosome number of N=12 including one h-chromosome, but they made no description on it.

During female prophase I the chromosomes were also visible along the paired

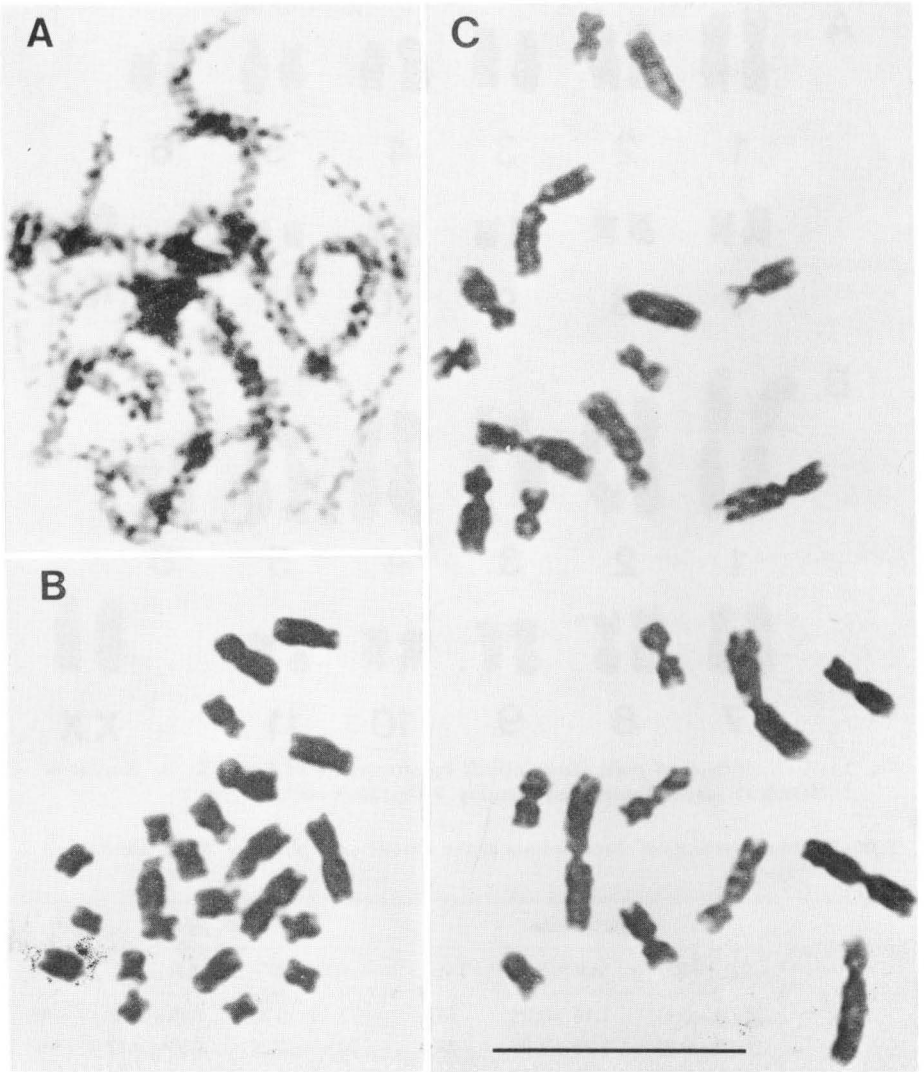

Fig. 8. Chromosomes of *Nerita albicila*. A. Female meiotic prophase I (zygotene): No h-chromosome appears; B. Spermatogonial metaphase (2N=23); C. Oogonial metaphase (2N=24). Scale bar: 10 μm.

homologues (Fig. 8-A), but not any h-chromosomes could be found. Unfortunately as I failed to find cells at the late meiotic stages, i.e. diakinesis or metaphase I, it is still unknown whether the negative heteropycnosis might appear at these stages.

The mitotic metaphase chromosomes were observed in both sexes and the diploid chromosome numbers of 23 for the male and 24 for the female were counted (Fig. 8-B & C). Fig. 9-A and B show the karyotypes of this species arranged by size. The larger 6 pairs of chromosomes were M or SM and the smaller 5 pairs are M. There was one non-paired T chromosome recognized easily in male complements, whereas there was a duplication of this element in females, which presumably

Fig. 9. A. Karyotype of male *Nerita albicila* constructed from Fig. 8-B; B. Karyotype of female *N. albicila* constructed from Fig. 8-C. Scale bar: 10 μm.

Table 5. Measurements of chromosomes at the mitotic metaphase of *Nerita albicila* and *N. japonica*.

Chromosome	*Nerita albicila*			*N. japonica*		
	RL±SE	AR±SE	Type	RL±SE	AR±SE	Type
1	14.00±0.41	1.16±0.03	M	13.59±0.37	1.15±0.01	M
2	11.79±0.35	1.25±0.06	M	10.48±0.27	1.53±0.12	M
3	11.68±0.20	2.27±0.16	SM	10.38±0.12	2.46±0.30	SM
4	10.11±0.33	1.35±0.07	M	10.00±0.05	1.24±0.06	M
5	9.11±0.24	2.46±0.32	SM	9.25±0.08	1.36±0.08	M
6	8.30±0.15	1.77±0.18	SM/M	9.09±0.11	1.82±0.13	SM/M
7	7.92±0.22	1.62±0.08	M	8.32±0.09	1.62±0.15	M
8	7.53±0.15	1.20±0.04	M	8.31±0.15	1.13±0.07	M
9	6.91±0.25	1.07±0.02	M	7.69±0.20	1.19±0.08	M
10	6.41±0.23	1.34±0.10	M	6.48±0.20	1.14±0.07	M
11	6.13±0.24	1.08±0.01	M	6.22±0.37	1.48±0.04	M
X*	7.56±0.58	—	T	14.08±0.31	2.14±0.15	SM
TCL	60.65±5.63			61.56±2.75		

*: ratio of the sex chromosome length to the total haploid autosome length (TCL) in percentage. See Table 3 for further explanations.

indicates these telocentrics to be sex-determining chromosomes (X-chromosomes). The mean of the total autosome length in the diploid was 60.65 ± 5.63 μm and that of the X-chromosome length was 2.30 ± 0.21 μm. Observed autosomes ranged from 1.36 to 6.79 μm in length. Chromosome measurements are shown in Table 5.

7. *Nerita helicinoides laevilabris* Pilsbry, 1895

Though meiosis in males was observed, I could not find cells at prophase I. At metaphase I, 12 chromosomes, including one h-chromosome which stained weakly and was less condensed, a negative heteropycnotic, were observed (Fig. 10-A). This number coincides with the report of Komatsu & Inaba (1982). Fig. 10-B shows a metaphase II chromosome plate, which contained 12 chromosomes without any special chromosomes. I previously observed the chromosomes at metaphase II of *N. lineata*, and reported no special chromosomes there (Nakamura, 1985a).

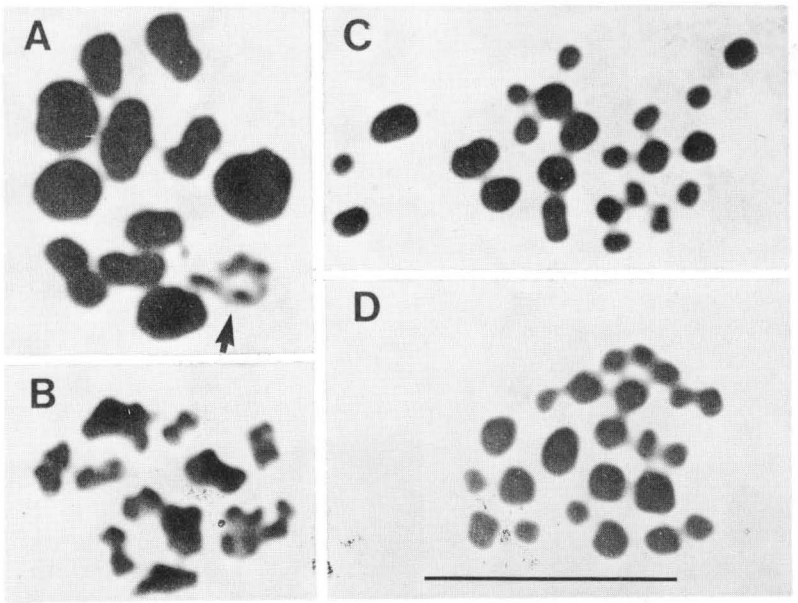

Fig. 10. A–B. Meiosis in male *Nerita helicinoides laevilabris*. A. Metaphase I (N=12, 11+h). An arrow indicates the h-chromosome; B. Metaphase II (N=12). No h-chromosome appears. C–D. Spermatogonial metaphase chromosomes of *N. insculpta* (N=23). Scale bar: 10 μm.

The h-chromosome at metaphase I of this species closely resembles those of some Hong Kong neiritids (Nakamura, 1985a), and therefore this can be a univalent and correspond to the non-paired X-chromosome in the male diploid. This h-chromosome is presumably related to sex determination. Consequently this species is expected to have 23 chromosomes in the male diploid.

8. *Nerita insculpta* Reculz, 1842

Twenty-three chromosomes were counted at spermatogonial metaphase (Fig.

10-C & D). They were so strongly condensed perhaps as a result of over-treatment with colchicine that I could not give a detailed description of their morphology. Concerning the odd number of chromosomes in the diploid, this species is expected to have at least one non-paired X-chromosome. This would be also supported by the observations on this species by Komatsu & Inaba (1982): they found one h-chromosome at male meiotic prometaphase I.

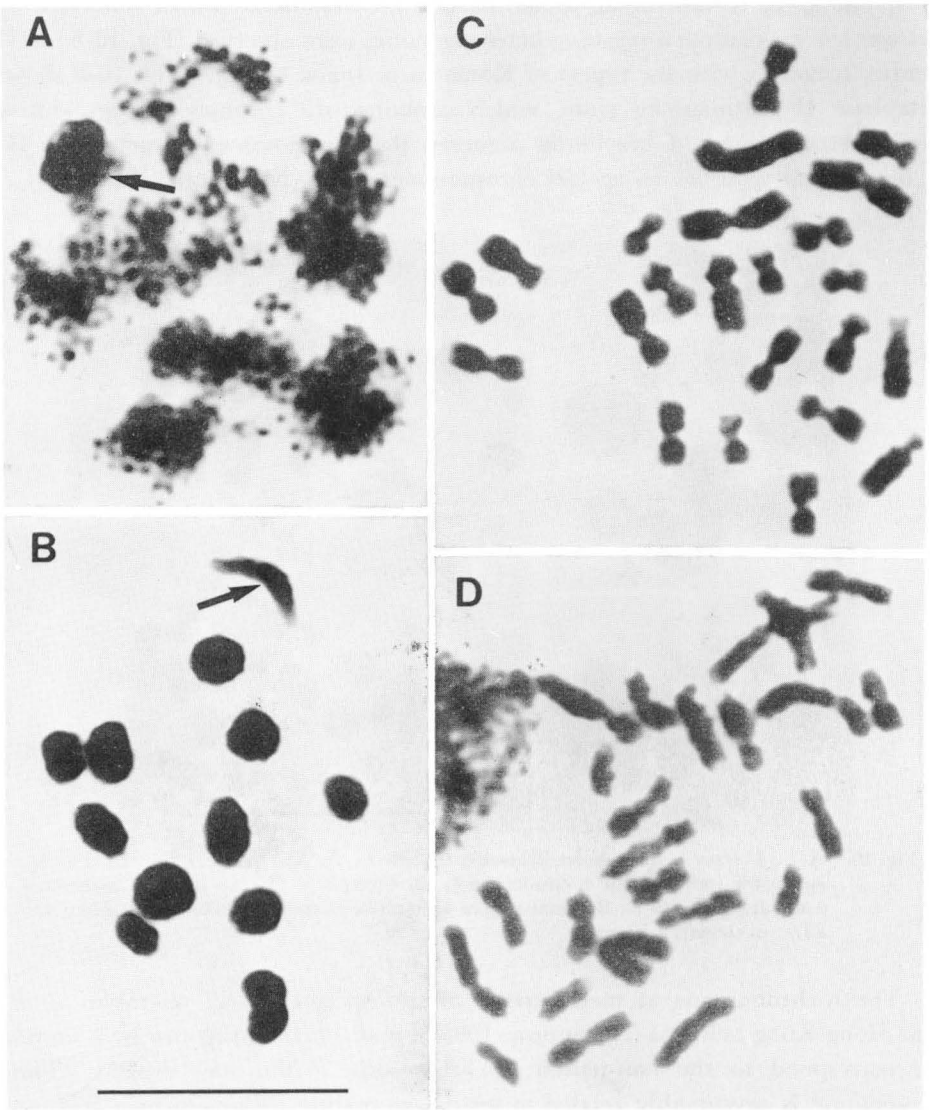

Fig. 11. Chromosomes of *Nerita japonica*. A–B: Male meiosis. C–D: Mitosis. A. Prophase I (leptotene): all the chromosomes except the single condensed h-chromosome (arrowed) are long, fine threads; B. Metaphase I (N=12, 11+h) (An arrow indicates the h-chromosome); C. Spermatogonial metaphase (2N=23); D. Oogonial metaphase (2N=24). Scale bar: 10 μm.

9. *Nerita japonica* Dunker, 1860

At the early male meiosis, the h-chromosome appeared to be a compact dark mass, the heteropycnotic chromosome, whereas the others were fine long threads (Fig. 11-A). All the chromosomes except the h-chromosome took more or less elliptical or oval shape at metaphase I (Fig. 11-B). The h-chromosome was less contracted, or negatively heteropycnotic at this stage. A small contraction of the h-chromosome could be recognized but not so clear as observed in some neritids from Hong Kong (Nakamura, 1985a). Twelve chromosomes including one h-chromosome could be observed at this stage. Komatsu & Inaba (1982) reported the same chromosome number of N=12 including one h-chromosome in male meiosis of this species. Judging from their photograph, as they gave no descriptions of this special chromosome, the h-chromosome seems to have the same appearance as the one that I observed in the present study. Nishikawa (1962), however, reported 11 haploid chromosomes in male metaphase I and II of this species. I found two kinds of meiotic chromosome plates in the metaphase II of *N. lineata* (Nakamura, 1985a): one containing only autosomes, N=11, and the other containing autosomes and the X-chromosome, thus N=12, making a different count in meiosis. Nishikawa may have observed such a metaphase II plate lacking the X-chromosome. Alternatively, as noted by Patterson (1967b), it may be due to the inadequencies of Nishikawa's old sectioning method that he could not identify the sex chromosome.

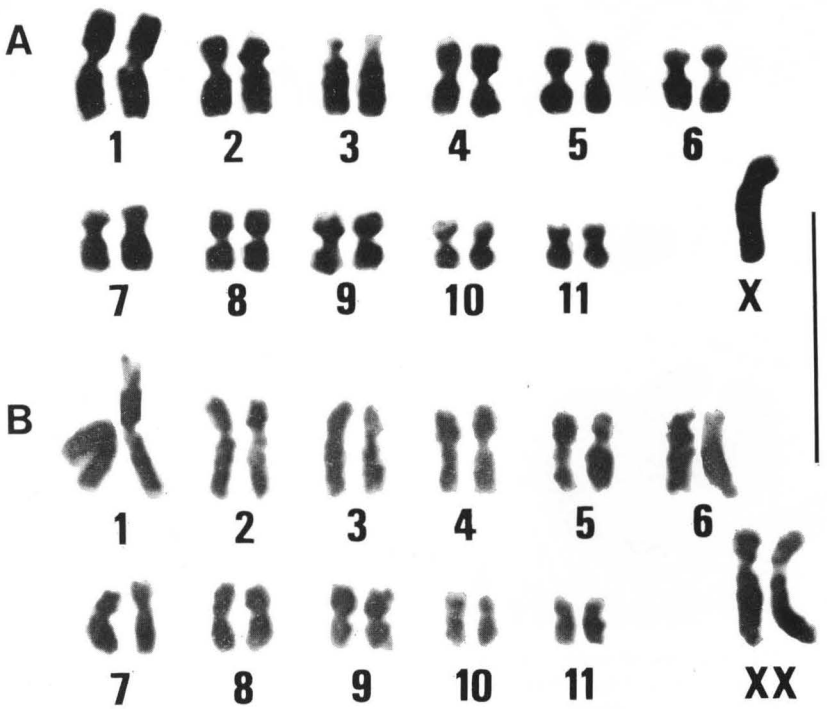

Fig. 12. A. Karyotype of male *Nerita japonica* constructed from Fig. 11-C; B. Karyotype of female *N. japonica* constructed from Fig. 11-D. Scale bar: 10 μm.

The mitotic metaphase chromosomes were observed in both sexes and the diploid chromosome numbers of 23 for males and 24 for females were counted (Fig. 11-C & D). The karyotype of this species consisted of one large M, one typical SM and the remaining nine pairs of mostly M chromosomes (Fig. 12-A & B), which coincides with the results of Komatsu & Inaba (1982) on the males of this species although their method differed from mine. There was one non-paired large SM in males and a duplication of this element in females, and therefore these were probably X-chromosomes. The X-chromosome was the largest in the complements. The mean of the total autosome length was $61.56 \pm 2.75\ \mu m$ and that of the X-chromosome was $4.54 \pm 0.25\ \mu m$. Observed autosomes ranged from 1.31 to $5.80\ \mu m$ in length. Chromosome measurements are shown in Table 5.

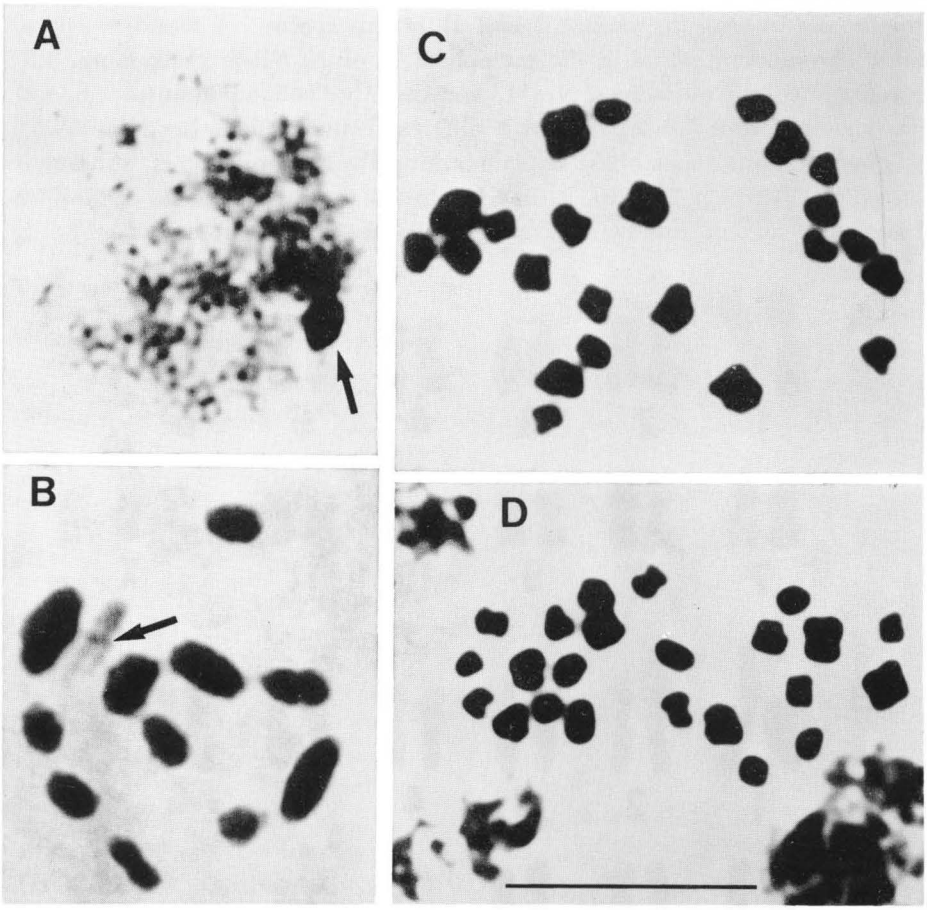

Fig. 13. Chromosomes of male *Nerita plicata*. A–B: Meiosis. C–D: Mitosis. A. Prophase I (leptotene). (An arrow indicates the h-chromosome); B. Metaphase I (N=12, 11+h): The h-chromosome is clearly showing two chromatids (i.e., a univalent) with a small contraction (arrowed); C, D. Spermatogonial metaphase (2N=23). Scale bar: $10\ \mu m$.

10. *Nerita plicata* Linnaeus, 1758

The chromosomes in male meiosis were observed. An h-chromosome was clearly distinguishable as a condensed mass during prophase I (Fig. 13-A). All the chromosomes except the h-chromosome were contracted at metaphase I (Fig. 13-B). The h-chromosome was less condensed, with a small contraction like a node. Twelve chromosomes including one h-chromosome could be counted at this stage. Komatsu & Inaba (1982) reported a chromosome number of N=12 at male prometaphase I and found one of them was heterochromatic. They gave no descriptions of it, but in their photograph the h-chromosome appears similar to what I observed in the present study.

Twenty-three chromosomes were counted at spermatogonial metaphase (Fig.

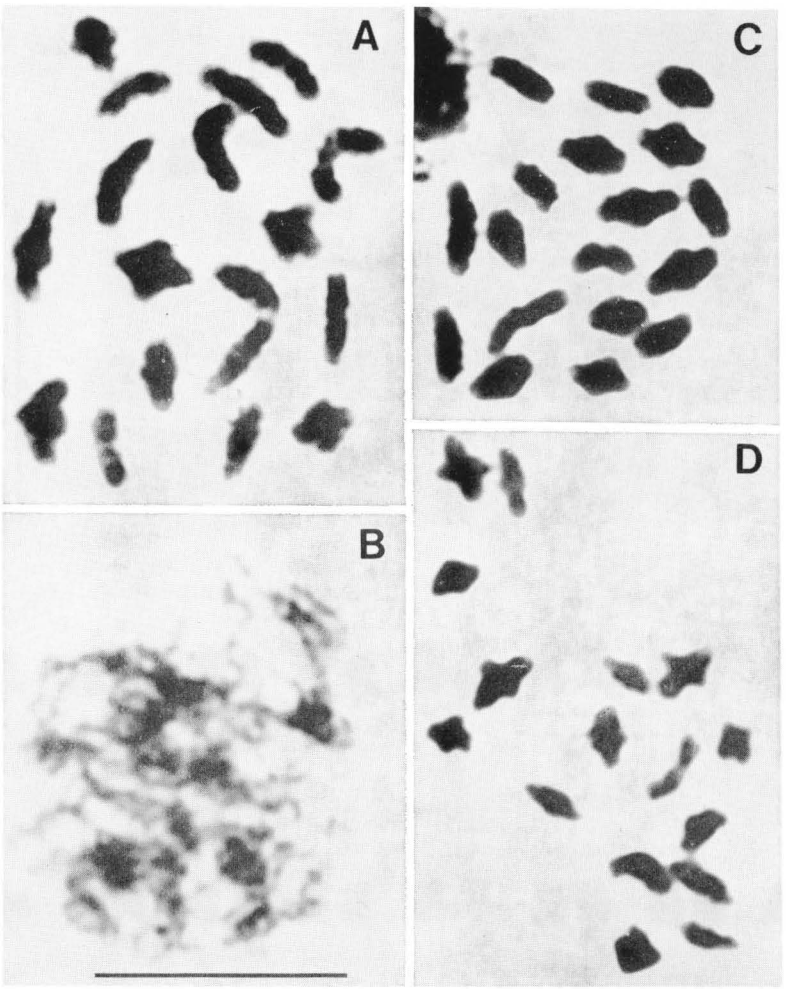

Fig. 14. A–B. Meiosis in male *Haliotis gigantea*. A. Diakinesis (N=18); B. Early metaphase I (N=18). C–D. Meiosis in male *H. varia*. C. Prophase I (leptotene); D. Diakinesis (N=16). Scale bar: 10 μm.

13-C & D). Unfortunately I could not obtain any suitable chromosome spreads to investigate morphology. Considering the odd number of chromosomes in the diploid, this species can also be expected to have at least one non-paired X-chromosome as shown previously for *N. albicila* and *N. japonica*.

Haliotidae (Haliotacea)

11. *Haliotis gigantea* Gmelin, 1791

Only the male meiotic cells were observed. Eighteen chromosomes could be counted at diakinesis and metaphase I (Fig. 14-A & B). There were no h-chromosomes observed during the meiotic stages. This chromosome number is the same as that of *H. discus discus, H. d. hannai* (Arai et al., 1982) and an American abalone, *H.*

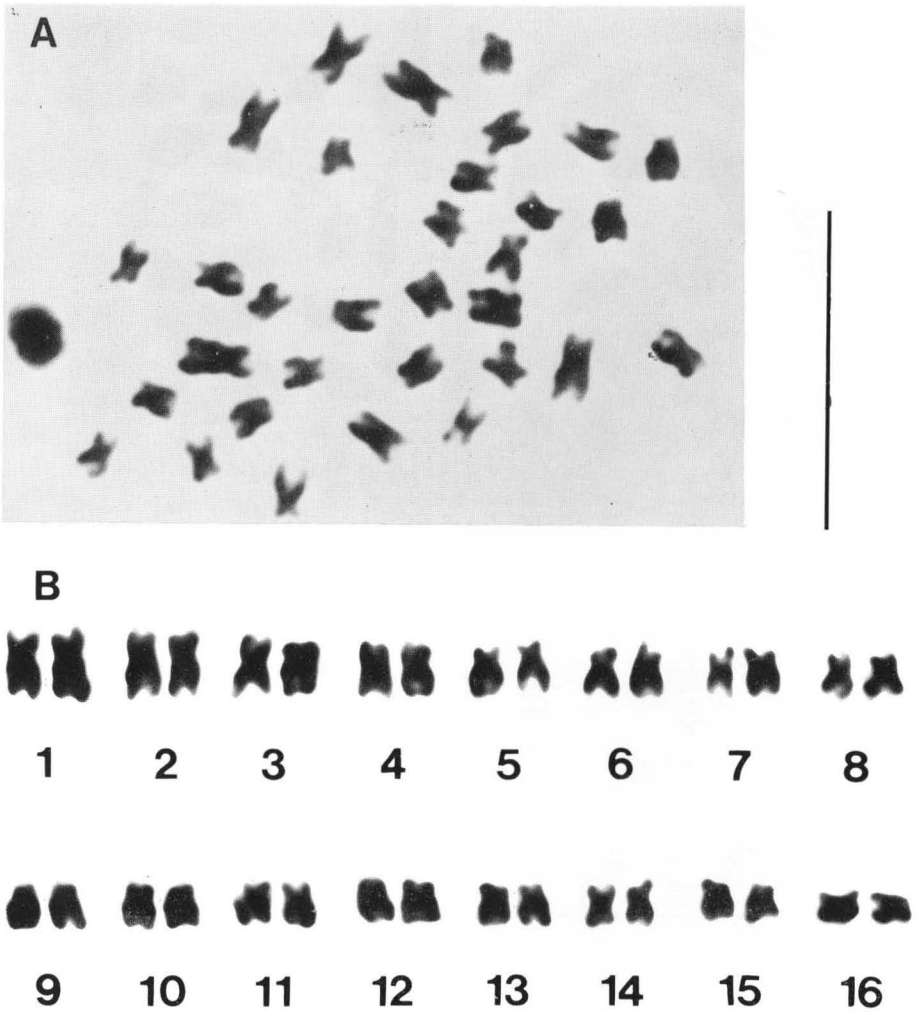

Fig. 15. Chromosomes of *Haliotis varia*. A. Spermatogonial metaphase (2N=32); B. Karyotype constructed from Fig. 15-A. Scale bar: 10 μm.

Table 6. Measurements of chromosomes at the mitotic metaphase of *Diodora quadriradiatus*, *Tugali decussata*, *Macroschisma dilatata* and *Montfortula pulchra picta*.

Chromosome	*Diodora quadriradiatus*			*Tugali decussata*		
	RL±SE	AR±SE	Type	RL	AR	Type
1	9.78±0.34	2.35±0.11	SM	9.66	1.29	M
2	9.11±0.30	2.29±0.23	SM	9.45	1.26	M
3	8.51±0.07	1.62±0.03	M	8.84	1.76	SM
4	7.59±0.11	2.17±0.11	SM	7.73	1.98	SM
5	7.01±0.09	2.34±0.11	SM	7.63	1.17	M
6	6.54±0.11	1.78±0.12	SM/M	7.37	1.43	M
7	6.42±0.11	3.05±0.19	ST/SM	6.95	2.27	SM
8	6.31±0.11	3.09±0.17	ST/SM	6.38	1.09	M
9	5.88±0.16	2.87±0.13	SM/ST	6.21	1.65	M
10	5.50±0.09	1.55±0.16	M/SM	5.71	1.25	M
11	5.16±0.13	2.02±0.12	SM	5.61	1.22	M
12	5.16±0.10	7.92±0.80	T/ST	5.06	2.20	SM
13	4.83±0.16	1.38±0.13	M	4.70	1.22	M
14	4.66±0.16	3.00±0.20	ST/SM	4.47	2.53	SM
15	3.85±0.04	1.40±0.05	M	4.20	1.56	M
16	3.83±0.14	2.36±0.24	SM	—	—	—
TCL	83.74±1.29			68.47		

Chromosome	*Macroschisma dilatata*			*Montfortula pulchra picta*		
	RL±SE	AR±SE	Type	RL±SE	AR±SE	Type
1	8.10±0.09	1.74±0.10	SM/M	11.02±0.26	1.19±0.10	M
2	7.45±0.27	1.66±0.14	M/SM	10.12±0.20	1.25±0.06	M
3	7.33±0.05	1.21±0.12	M	8.64±0.21	1.16±0.04	M
4	7.11±0.07	1.25±0.06	M	8.03±0.18	1.18±0.05	M
5	6.90±0.06	2.10±0.13	SM	7.52±0.12	1.68±0.20	M/SM
6	6.81±0.15	1.17±0.02	M	7.31±0.12	1.59±0.10	M/SM
7	6.60±0.04	1.32±0.09	M	6.91±0.16	1.15±0.03	M
8	6.40±0.08	1.25±0.07	M	6.34±0.14	1.22±0.06	M
9	6.27±0.09	1.11±0.02	M	6.23±0.10	1.24±0.05	M
10	6.15±0.19	1.17±0.02	M	6.03±0.09	1.61±0.17	M/SM
11	6.00±0.07	1.79±0.16	SM/M	5.97±0.13	1.17±0.03	M
12	5.70±0.04	1.56±0.05	M	5.50±0.13	1.18±0.07	M
13	5.55±0.13	1.22±0.10	M	5.44±0.06	1.50±0.10	M/SM
14	4.88±0.27	1.13±0.05	M	4.93±0.15	1.19±0.06	M
15	4.54±0.28	1.67±0.05	M/SM	—	—	—
16	4.07±0.23	3.69±0.19	ST	—	—	—
TCL	64.21±0.59			58.36±0.96		

See Table 3 for explanations.

cracherodii (Minkler, 1977).

12. *Haliotis varia* Linnaeus, 1758

Meiosis in males was observed. During prophase I the chromosomes were in a bouquet arrangement which was gradually lost as chromosome contraction proceeded (Fig. 14-C). Sixteen chromosomes could be observed at diakinesis and metaphase I (Fig. 14-D). No h-chromosome was found during male meiotic stages.

Thirty-two chromosomes were observed at spermatogonial metaphase (Fig. 15-A). Fig. 15-B shows the karyotype of this species tentatively arranged by size, for the smaller chromosomes were very similar both in size and shape, and it was very difficult to pair them. The majority of the chromosomes seems to be M, and Nos. 5, 6 and 9 pairs to be SM. Mean total chromosome length of five chromosome sets in the diploid is $65.39 \pm 3.19\,\mu$m. In general, observed chromosomes are rather short in size, varying gradually from 1.41 to 3.63 μm.

Though the chromosome number is the same between this species and *H. aquatilis* (Nakamura, 1985b), the karyotype is different. *H. aquatilis* has 5 pairs of SM, 2 SM/ST and 1 typical ST chromosomes, whereas *H. varia* does not have any ST chromosomes.

Fissurellidae (Fissurellacea)

13. *Diodora quadriradiatus* (Reeve, 1850)

Only mitotic spreads were found. Thirty-two chromosomes were counted at spermatogonial metaphase (Fig. 16-A). The karyotype of this species consisted of mostly heterobranchial chromosomes, i.e. SM, ST and T chromosomes (Fig. 16-B). Typical M chromosomes were found only in three pairs. All the chromosomes were rather large and mean total chromosome length in the diploid is $83.74 \pm 1.29\,\mu$m. Observed chormosomes ranged from 1.37 to 4.82 μm in length. Chromosome measurements are shown in Table 6.

14. *Tugali decussata* A. Adams, 1852

Fifteen bivalents were observed at the metaphase I both in males (Fig. 17-A) and females (Fig. 17-B). No sexual differences was found between them. As I could not find any spermatogonial metaphase spreads, I used oogonia and observed 30 chromosomes at oogonial metaphase (Fig. 17-C). All the chromosomes were M or SM, and two of them were clearly larger (Fig. 17-D). Only one set of the chromosomes shown in Fig. 17-C was measured and the results were shown in Table 6. Chromosomes ranged from 1.44 to 3.50 μm in length, and the total length was 68.47 μm.

15. *Macroschisma dilatata* A. Adams, 1851

Sixteen bivalents in the first spermatocyte (Fig. 18-A) and 32 chromosomes at spermatogonial metaphase (Fig. 18-B) were counted, which confirmed Nishikawa's previous counting (Nishikawa, 1962). The karyotype of this species consisted of all

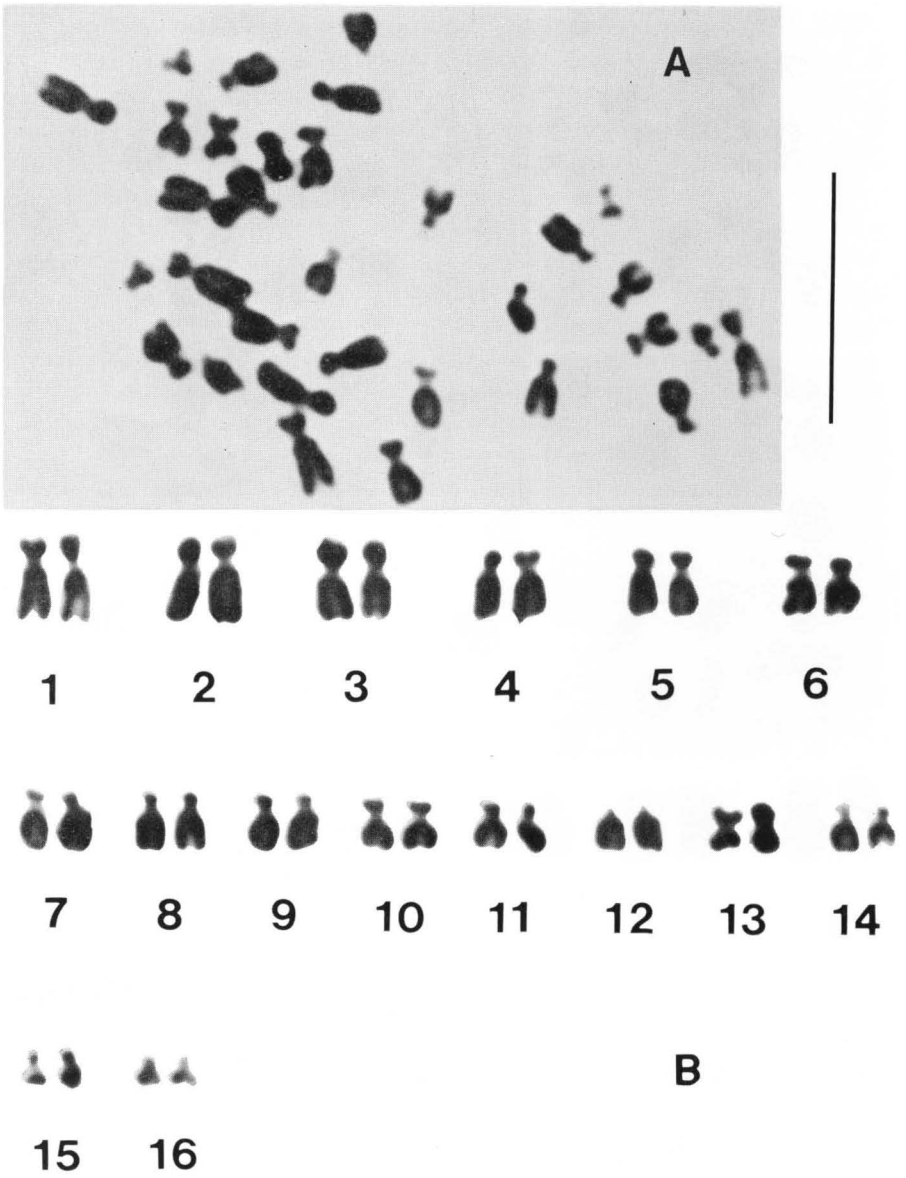

Fig. 16. Chromosomes of *Diodora quadriradiatus*. A. Spermatogonial metaphase (2N=32). B. Karyotype constructed from Fig. 16-A. Scale bar: 10 μm.

M or SM except the smaller pair of T chromosomes (Fig. 18-C). Mean length of total diploid chromosomes was $64.21 \pm 0.59\ \mu$m. In general, the chromosomes were shorter, varying gradually from 1.11 to 2.78 μm in length. Chromosome measurements are shown in Table 6.

16. *Montfortula pulchra picta* (Dunker, 1860)

I observed 14 bivalents in the first spermatocyte (Fig. 19-A), which is very

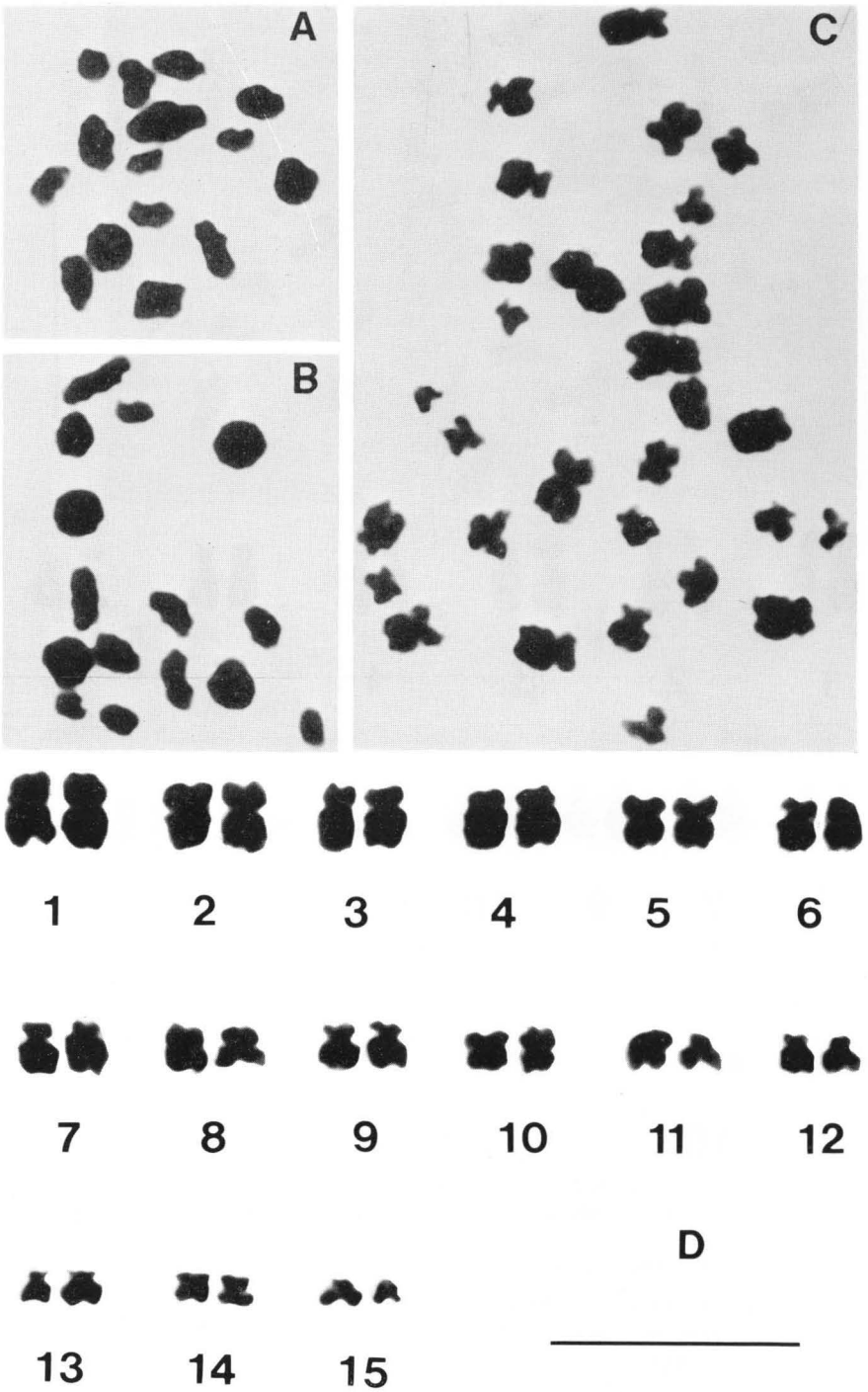

Fig. 17. Chromosomes of *Tugali decussata*. A–B: Meiosis. C–D: Female mitosis. A. Male metaphase I (N=15); B. Female metaphase I (N=15); C. Oogonial metaphase (2N=30); D. Karyotype constructed from Fig. 17-C. Scale bar: 10 μm.

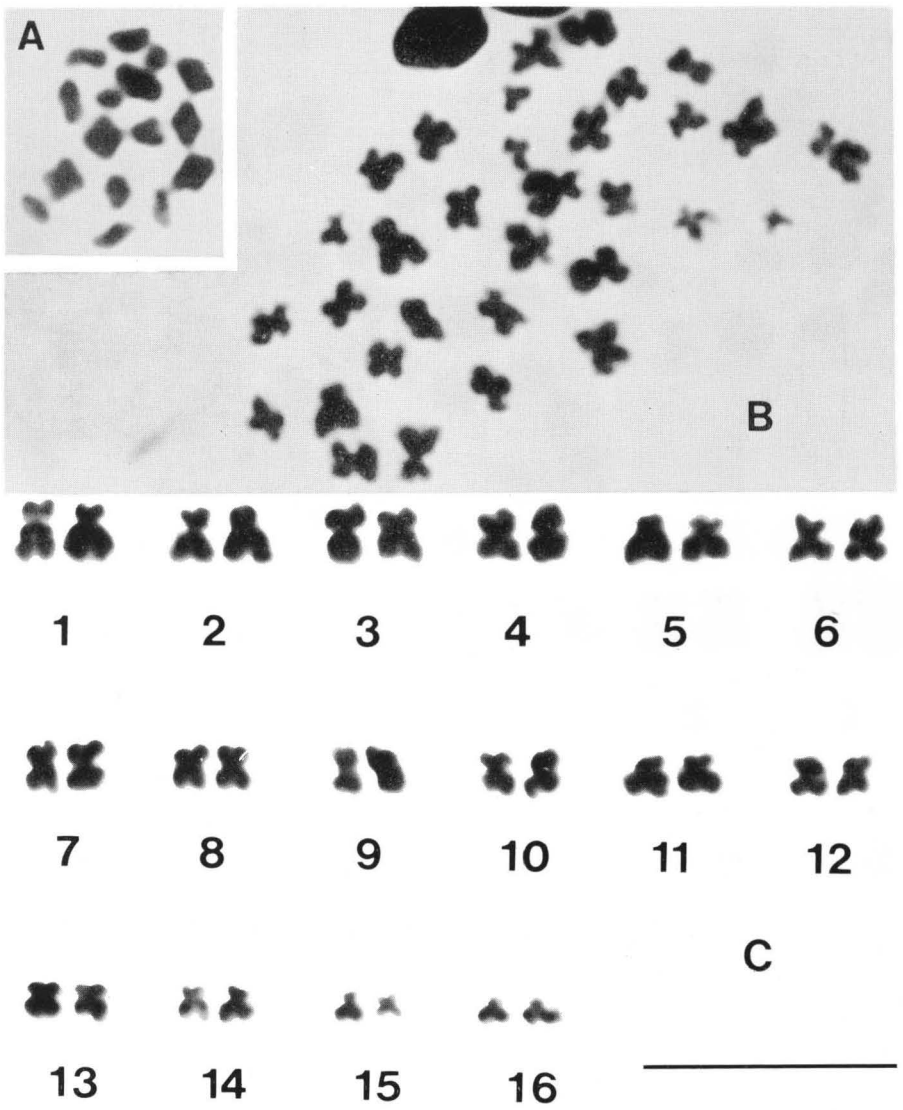

Fig. 18. Chromosomes of male *Macroschisma dilatata*. A. Meiotic metaphase I (N=16); B. Spermatogonial metaphase (N=32); C. Karyotype constructed from Fig. 18-B. Scale bar: 10 μm.

different from the result of Nishikawa's observation (Nishikawa, 1962); he reported 17 bivalents in meiosis.

Several investigators have reported the results which are different from Nishikawa's countings. Nishikawa's cytological technique failed to reveal small chromosomes (for example, in Acmaeidae: Nakamura, 1982a, b), or resulted in repetition of chromosome count (for example, in the mesogastropod Cerithidae: Komatsu, 1985; and in Haliotidae: Nakamura, 1985b). As his work was based mainly on metaphase I chromosomes using the old sectioning method, what has been counted

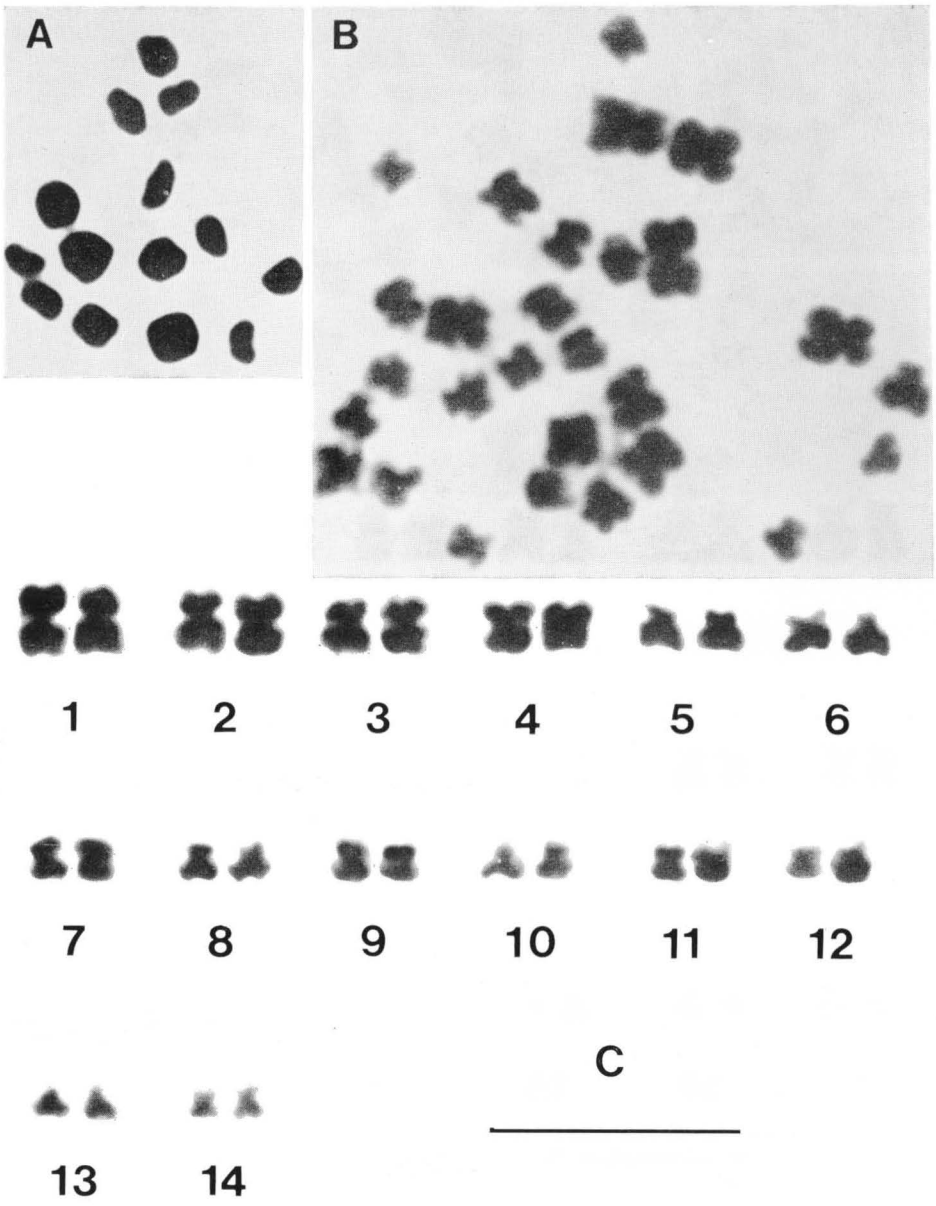

Fig. 19. Chromosomes of male *Montfortula pulchra picta*. A. Meiotic metaphase I (N=14); B. Spermatogonial metaphase (2N=28); C. Karyotype constructed from Fig. 19-B. Scale bar: 10 μm.

are visible bodies. No doubt most are bivalents but in some cases they are possibly univalents or multivalents, rather than single chromosomes. This may explain the difference observed here. Neverthless the difference is still very remarkable. Nishikawa may have examined different species. Another explanation for the difference is that this species might have intraspecific polymorphism in chromosome number.

However, I have never observed such a condition at least in the local population around the laboratory. I observed 28 chromosomes at spermatogonial metaphase (Fig. 19-B), which consisted of all M or SM chromosomes. Two were clearly larger M pairs and the others sharply decreased in size (Fig. 19-C). Mean total chromosome length in the diploid was $58.36 \pm 0.96\ \mu m$. Observed chromosomes ranged from 1.25 to 3.50 μm in length. Chromosome measurements are shown in Table 6.

Trochidae (Trochacea)

17. *Monodonta australis* Lamarck, 1818

A chromosome number of 2N=36 was counted at spermatogonial metaphase (Fig. 20-A). Chromosomes were very much condensed in most cells (Fig. 20-B), and could not be morphologically analyzed. Some spreads, however, indicated that 6 of the chromosomes were apparently ST and the others seemed to be SM or M chromosomes (Fig. 20-A). There were no meiotic chromosomes found in the

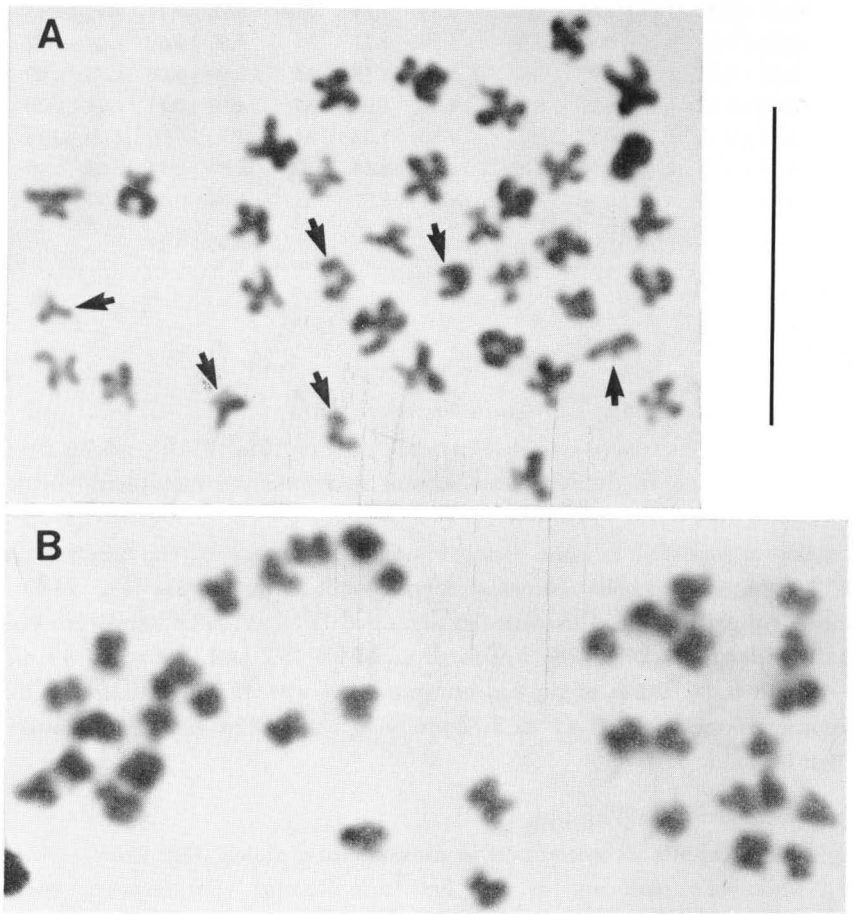

Fig. 20. Spermatogonial metaphase chromosomes of *Monodonta australis* (2N=32). A. Arrows indicate subtelocentric chromosomes; B. Condensed chromosomes. Scale bar: 10 μm.

Table 7. Measurements of chromosomes at the mitotic metaphase of *Monodonta labio confusa*, *M. neritoides* and *Omphalius nigerrima*.

Chromosome	*Monodonta labio confusa*			*Omphalius nigerrima*			*M. neritoides*		
	RL±SE	AR±SE	Type	RL	AR	Type	RL±SE	AR±SE	Type
1	6.93±0.10	1.46±0.07	M	6.64	1.34	M	7.22±0.15	1.15±0.04	M
2	6.41±0.13	1.47±0.08	M	6.44	1.15	M	6.64±0.31	1.16±0.08	M
3	6.40±0.19	1.94±0.11	SM	6.12	2.25	SM	6.43±0.10	1.25±0.09	M
4	6.20±0.19	3.10±0.34	ST/SM	6.06	1.04	M	6.28±0.08	1.55±0.09	M/SM
5	6.18±0.09	1.34±0.07	M	5.94	1.24	M	6.08±0.07	1.26±0.06	M
6	5.82±0.07	1.62±0.10	M/SM	5.88	2.05	SM	5.74±0.18	1.20±0.07	M
7	5.79±0.10	1.33±0.07	M	5.77	1.83	SM	5.73±0.16	1.64±0.08	M/SM
8	5.58±0.14	2.69±0.19	SM/ST	5.69	1.19	M	5.70±0.07	1.19±0.04	M
9	5.56±0.04	1.70±0.11	SM/M	5.63	1.49	M	5.47±0.14	1.14±0.03	M
10	5.35±0.10	3.08±0.17	ST/SM	5.56	1.38	M	5.38±0.06	3.44±0.26	ST
11	5.35±0.08	1.88±0.13	SM	5.44	1.27	M	5.31±0.05	1.55±0.03	M
12	5.35±0.04	1.27±0.06	M	5.39	1.24	M	5.20±0.11	3.35±0.15	ST
13	5.16±0.11	3.50±0.26	ST	5.29	1.11	M	5.08±0.09	1.18±0.02	M
14	5.12±0.12	1.34±0.07	M	5.09	1.18	M	5.00±0.08	1.36±0.09	M
15	5.06±0.06	1.90±0.08	SM	4.96	1.09	M	4.98±0.14	1.84±0.30	SM/M
16	4.67±0.14	1.42±0.12	M	4.80	1.33	M	4.91±0.06	1.89±0.14	SM/M
17	4.53±0.27	1.62±0.07	M/SM	4.75	2.55	SM	4.50±0.12	2.08±0.15	SM
18	4.38±0.10	3.53±0.20	ST	4.57	1.57	M	4.38±0.14	3.30±0.16	ST
TCL	84.66±4.18			89.37			80.46±6.77		

See Table 3 for explanations.

preparation.

18. *Monodonta labio confusa* Tapparone-Caneferi, 1874

Eighteen bivalents were counted in male meiosis (Fig. 21-A), which confirmed Nishikawa's counting in the first and second spermatocyte metaphase (Nishikawa, 1962).

Nishikawa reported neither number nor morphology of the mitotic chromosomes. I observed 36 chromosomes at spermatogonial metaphase (Fig. 21-B). Fig. 21-C shows the karyotype of this species arranged by size. The karyotype consisted of 13 pairs of larger M and SM, 3 of medium SM or ST and 2 of small ST chromosomes. Mean total length of diploid chromosomes was 84.66±4.18 μm. Observed chromosomes ranged from 1.47 to 3.72 μm in length. Chromosome measurements are shown in Table 7.

19. *Monodonta neriotides* (Philippi, 1850)

Eighteen bivalents were counted in male meiotic plates (Fig. 22-A), which confirmed Nishikawa's counting in the first and second spermatocyte metaphase (Nishikawa, 1962)

Thirty-six chromosomes were observed at spermatogonial metaphase (Fig. 22-

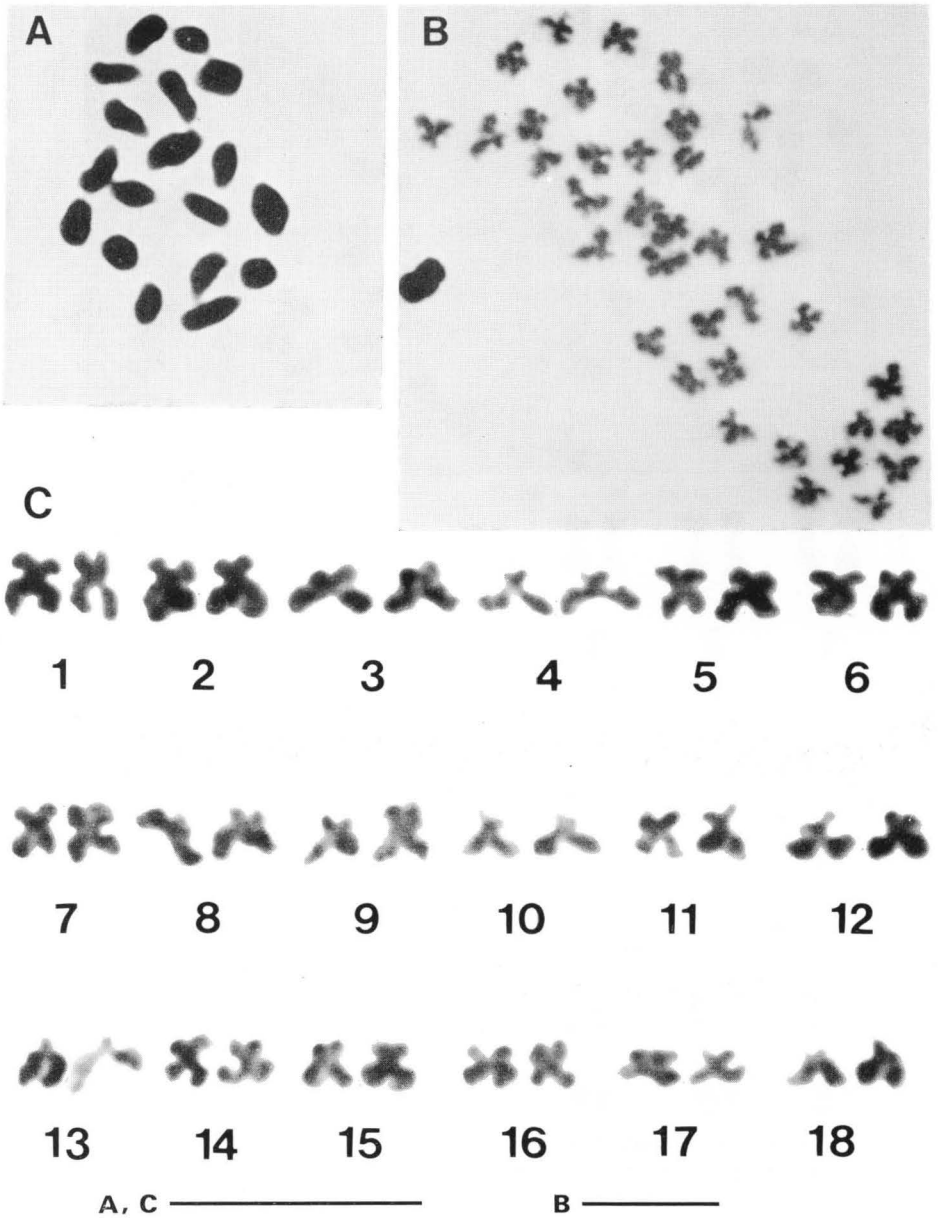

Fig. 21. Chromosomes of male *Monodonta labio confusa*. A. Meiotic metaphase I (N=18); B. Spermatogonial metaphase (2N=36); C. Karyotype constructed from the plate shown in Fig. 21-B. Scale bar: 10 μm.

B), and Fig. 22-C shows the karyotype arranged by size. The chromosome complements were gradually decreasing in length from No. 1 pair. The karyotype consisted of 15 pairs of M or SM and 3 of ST chromosomes. Mean total chromosome length in the diploid was 80.46 ± 6.77 μm. Observed chromosomes ranged from 1.30

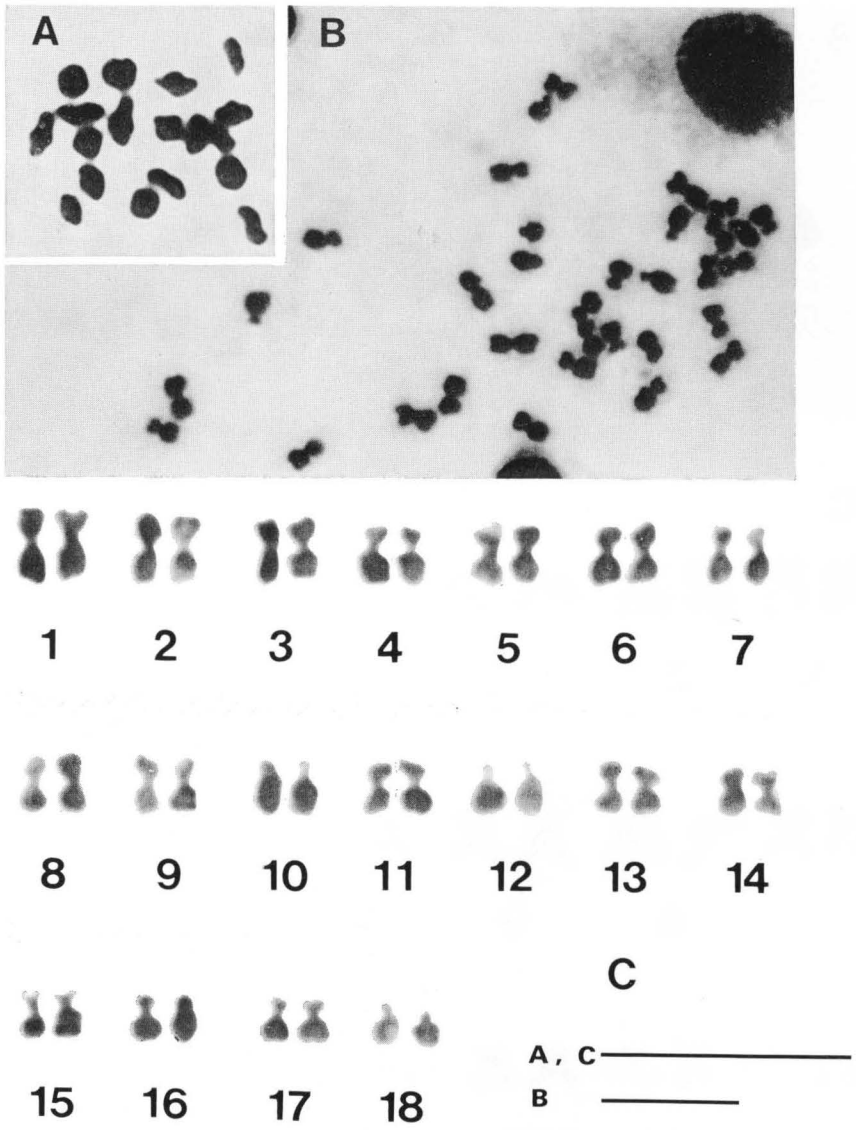

Fig. 22. Chromosomes of male *Monodonta neritoides*. A. Meiotic metaphase I (N=18); B. Spermatogonial metaphase (2N=36); C. Karyotype constructed from the plate shown in Fig. 22-B. Scale bar: 10 μm.

to 3.62 μm in length. Chromosome measurements are shown in Table 7.

20. *Monodonta perplexa* Pilsbry, 1889

Eighteen bivalents in the first spermatocyte (Fig. 23-B) and 36 chromosomes at spermatogonial metaphase were counted (Fig. 23-A), which are in accordance with the results of the chromosome number in other *Monodonta* species. Unfortunately the spermatogonial chromosomes were too condensed, probably as a result of over-treatment with colchicine, to permit description of their morphology.

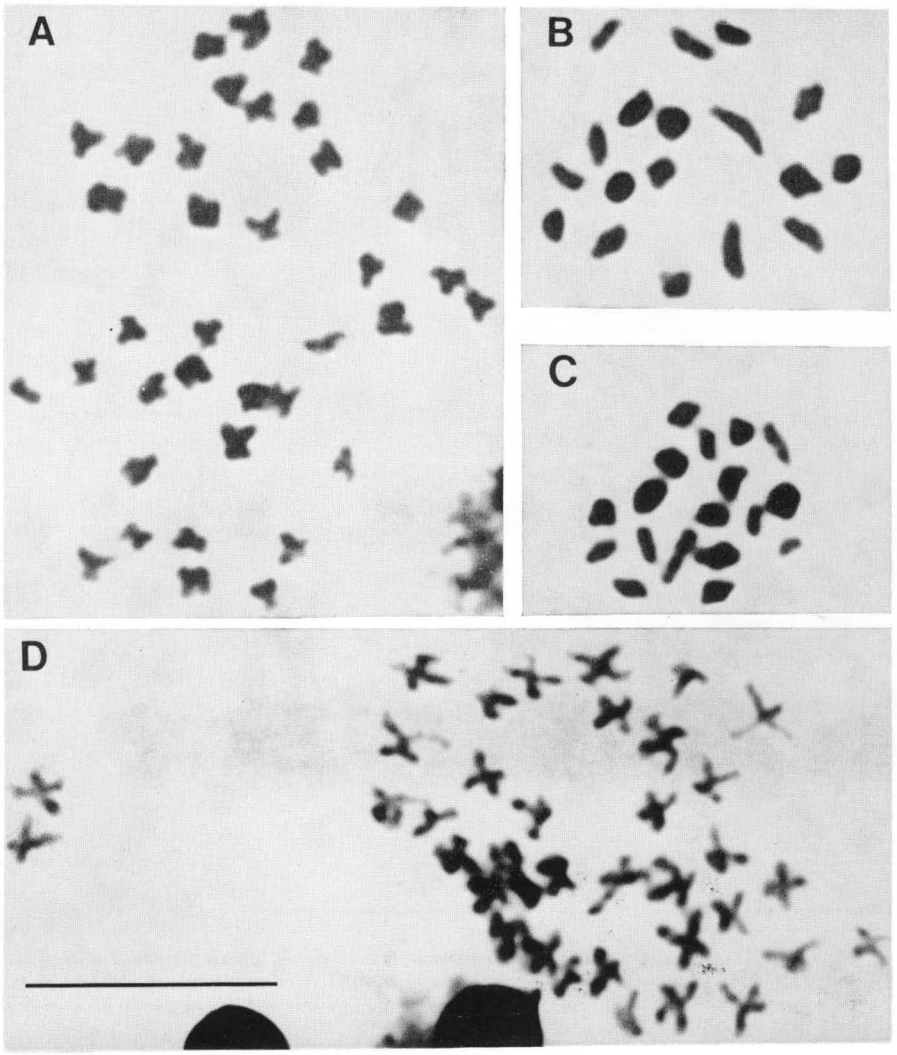

Fig. 23. A–B. Chromosomes of male *Monodonta perplexa*. A. Spermatogonial metaphase (2N =36); B. Meiotic metaphase I (N=18). C–D. Chromosomes of male *Pictodiloma suavis*. C. Meiotic metaphase I (N=18); D. Spermatogonial metaphase (2N= 36). Scale bar: 10 μm.

21. *Pictodiloma suavis* (Philippi, 1849)

Eighteen bivalents in the male meiotic plates (Fig. 23-C) and 36 chromosomes at spermatogonial metaphase (Fig. 23-D) were observed. The shapes and exact centromere positions of these chromosomes were rather unclear in all figures available. The smallest pair, however, was apparently ST, and one more ST pair was also observed; the rest of the complements seemed to be SM or M chromosomes.

22. *Chlorostoma argyrostoma lischkei* (Tapparone-Caneferi, 1874)

Eighteen bivalents were counted at the first meiotic metaphase in males (Fig.

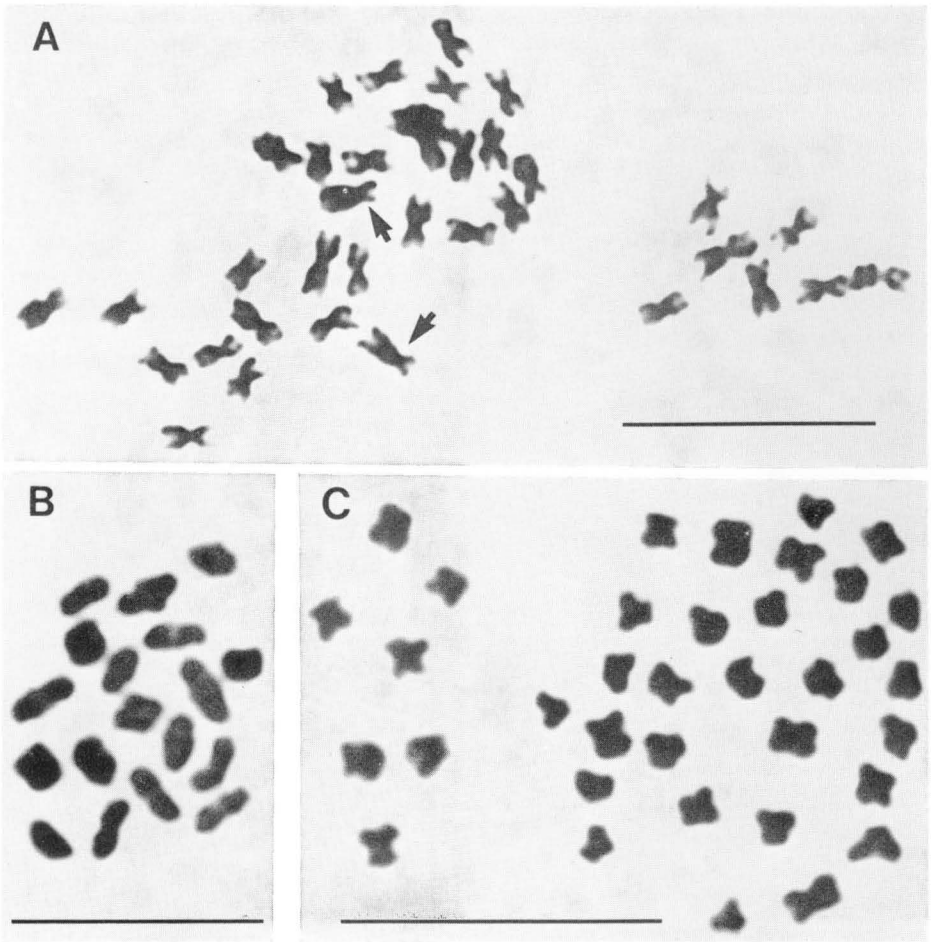

Fig. 24. A–B. Chromosomes of *Chlorostoma argyrostoma lischkei*. A. Spermatogonial metaphase (2N=36) (Arrows indicate relatively large SM/ST chromosomes); B. Male meiotic metaphase I (N=18). C. Spermatogonial metaphase chromosomes of *C. nigricolor* (2N=36). Scale bar: 10 μm.

24-B), which confirmed Nishikawa's early counting using the sectioning method (Nishikawa, 1962).

Only a few mitotic chromosome spreads were found; 36 chromosomes were counted in all of them (Fig. 24-A). The chromosomes condensed too much and were so small that their shapes and exact centromere positions were rather unclear except one pair of relatively large SM/ST chromosomes. There did not seem to be any typical ST or T chromosomes in the karyotype.

23. *Chlorostoma nigricolor* Dunker, 1860

Only a few dividing cells were found partly because their spermatogenesis was almost finished when I collected the specimens. Eighteen bivalents at metaphase I were counted (Fig. 25-A). In the spermatogonial metaphase spreads 36 chromosomes were observed (Fig. 24-C); unfortunately they were so condensed that I was

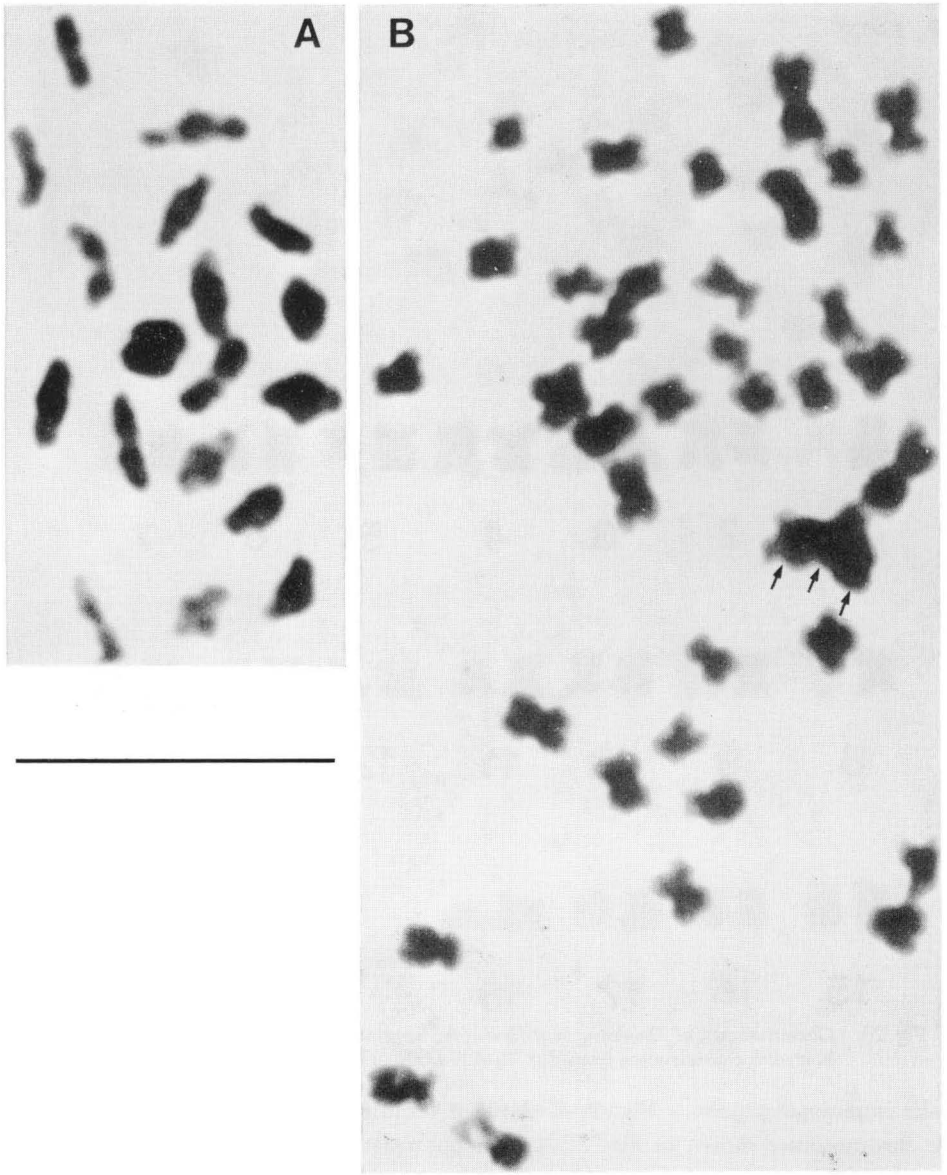

Fig. 25. A. Male meiotic metaphase I in *Chlorostoma nigricolor* (N=18). B. Spermatogonial metaphase chromosomes of *Granata lyrata* (2N=40) (Arrows indicate the region where three chromosomes stuck together). Scale bar: 10 μm.

unable to describe their morphology in detail.

24. *Omphalius nigerrima* (Gmelin, 1791)

Thirty-six chromosomes were counted at spermatogonial metaphase (Fig. 26-A). As I obtained only one suitable metaphase plate to investigate the morphology, and the medium size chromosomes were very similar in shape, it was very difficult to

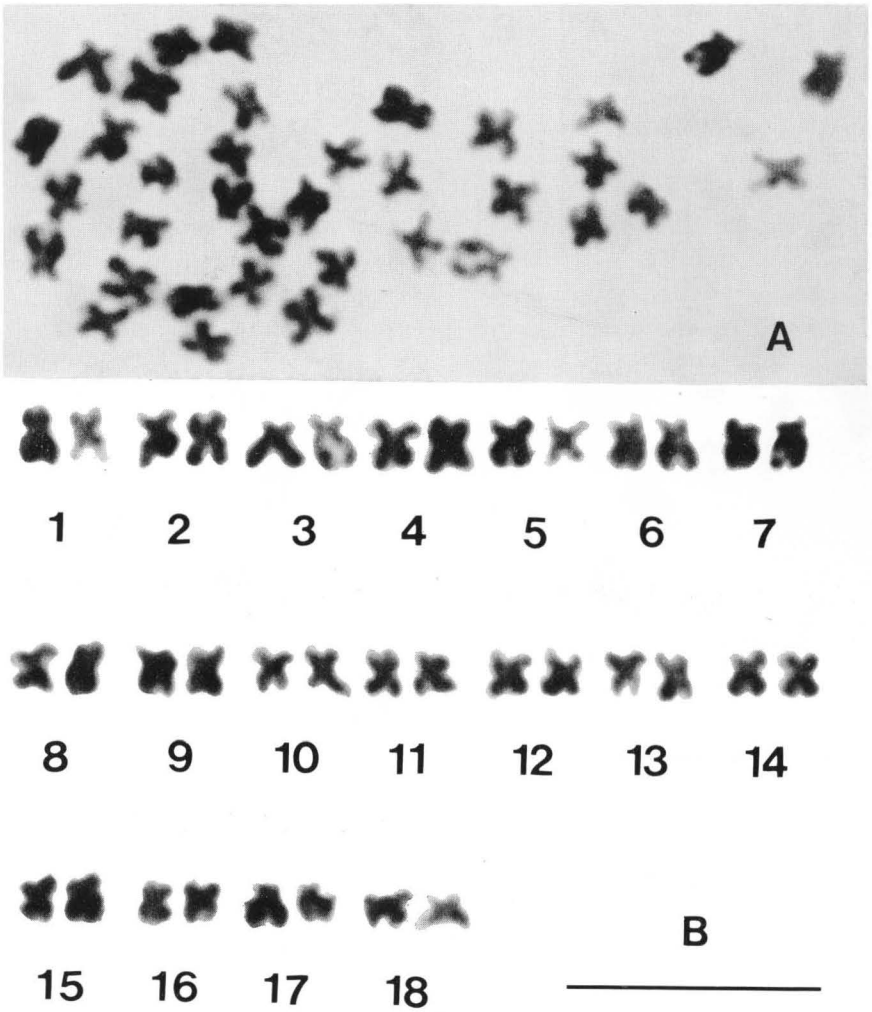

Fig. 26. Chromosomes of *Omphalius nigerrima*. A. Spermatogonial metaphase (2N=36). B. Karyotype constructed from Fig. 26-A. Scale bar: 10 μm.

make homologous pairs. Fig. 26-B shows a tentative karyotyping constructed from the chromosomes shown in Fig. 26-A, which consisted of 14 pairs of M and 4 of SM chromosomes. Total chromosome length in the diploid was 89.37 μm; the chromosomes ranged from 1.75 to 3.01 μm in length. Chromosome measurements are shown in Table 7.

Nishikawa (1962) reported 18 bivalents in the first spermatocyte cells, but he did not observe mitotic chromosomes.

25. *Granata lyrata* (Pilsbry, 1890)

There seemed to be 40 chromosomes in the spermatogonial spreads (Fig. 25-B) and 20 bivalents at early diakinesis. Only a few dividing cells could be found, and what was worse, they were in poor condition. Chromosome preparations and/or

staining procedures were not successful, and some of the chromosomes overlapped each other or stuck together so that it remains to be solved in future investigation if their chromosome number is exactly N=20 and 2N=40.

Nishikawa (1962) observed 21 dot-shaped bivalents of nearly equal size in the first spermatocyte metaphase. His observation is different from what I observed here in chromosome morphology as well as in chromosome number. I found 1 pair of relatively large M, 8–9 pairs of medium M or SM and the others of smaller chromosomes. Clearly, the chromosomes are not equal in size.

Nishikawa's early works were based mainly on sectioned material, which has often been indicated to produce artifacts. This might be the reason for the difference. However, it is not appropriate to neglect his count on this species as did Patterson (1967). Patterson's list did not report Nishikawa's result on this species, although her later list did (Patterson, 1969). I believe that this species has more than 18 chromosomes in the haploid and is quite distinct from other trochid snails in chromosome number.

Table 8. Measurements of chromosomes at the mitotic metaphase of *Lunella cornata coreensis* and *Broderipia iridescens*.

Chromosome	*Lunella coronata coreensis*			*Broderipia iridescens*		
	RL±SE	AR±SE	Type	RL	AR	Type
1	7.31±0.20	1.16±0.05	M	7.81	1.18	M
2	6.80±0.18	1.18±0.03	M	6.89	1.23	M
3	6.40±0.11	1.11±0.03	M	6.79	1.37	M
4	6.01±0.07	1.25±0.06	M	6.22	1.38	M
5	5.88±0.12	1.77±0.07	SM	6.08	1.21	M
6	5.86±0.06	1.31±0.06	M	6.05	1.09	M
7	5.70±0.06	1.20±0.05	M	5.89	1.30	M
8	5.57±0.06	1.22±0.05	M	5.79	1.18	M
9	5.53±0.08	2.07±0.11	SM	5.61	2.42	SM
10	5.50±0.07	1.29±0.05	M	5.44	3.08	ST
11	5.34±0.09	1.82±0.08	SM	5.43	1.33	M
12	5.29±0.05	1.23±0.04	M	5.12	1.50	SM
13	5.08±0.11	1.37±0.06	M	5.12	1.16	M
14	4.94±0.12	1.29±0.06	M	5.05	1.09	SM
15	4.88±0.15	3.31±0.20	ST	4.76	1.33	M
16	4.82±0.11	1.29±0.06	M	4.46	1.27	M
17	4.47±0.10	3.62±0.33	ST	4.00	2.58	SM
18	4.43±0.13	1.29±0.09	M	3.43	3.71	ST
TCL	83.53±3.03			95.75		

See Table 3 for explanations.

Turbinidae (Trochacea)

26. *Lunella coronata coreensis* Reculz, 1853

Eighteen bivalents were counted in male meiotic plates (Fig. 27-A), which

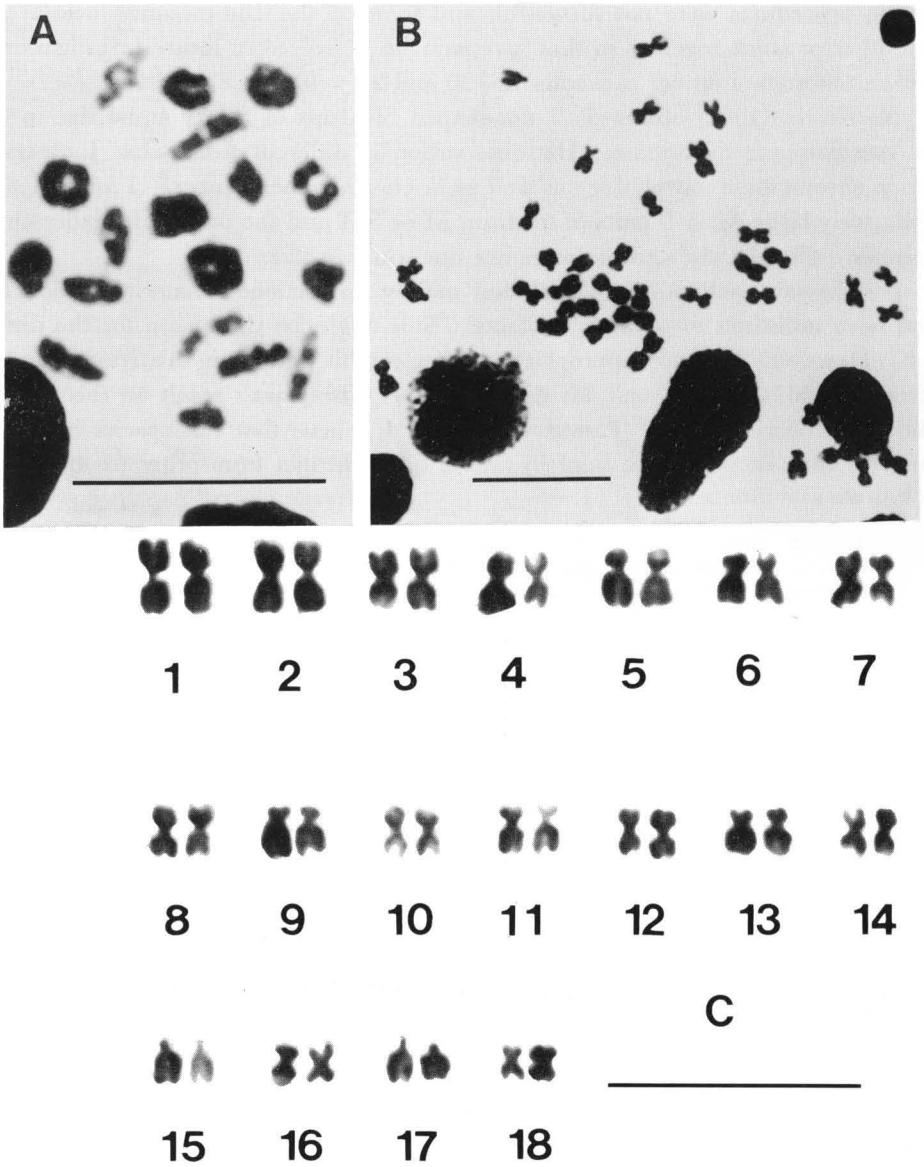

Fig. 27. Chromosomes of male *Lunella coronata coreensis*. A. Meiotic diakinesis (N=18); B. Spermatogonial metaphase (2N=36); C. Karyotype constructed from the plate shown in Fig. 27-B. Scale bar: 10 μm.

confirmed Nishikawa's earlier count (Nishikawa, 1962).

Thirty-six chromosomes were observed at spermatogonial metaphase (Fig. 27-B). Fig. 27-C shows the karyotype of this species arranged by size, which consisted of 13 pairs of M, 3 of which were remarkably larger, 3 of medium SM, and 2 of small ST chromosomes. Mean total chromosome length in the diploid was 83.53 $\pm$3.03 μm. Observed chromosomes ranged from 1.49 to 3.58 μm in length.

Chromosome measurements are shown in Table 8.

Komatsu (1984) reported chromosome number and centromere positions of this species, which very much coincided with the present observation in morphology and total length as well as in number of chromosomes although the applied methods were different, i.e. aceto-orcein squash method in Komatsu's work and warm-dry method

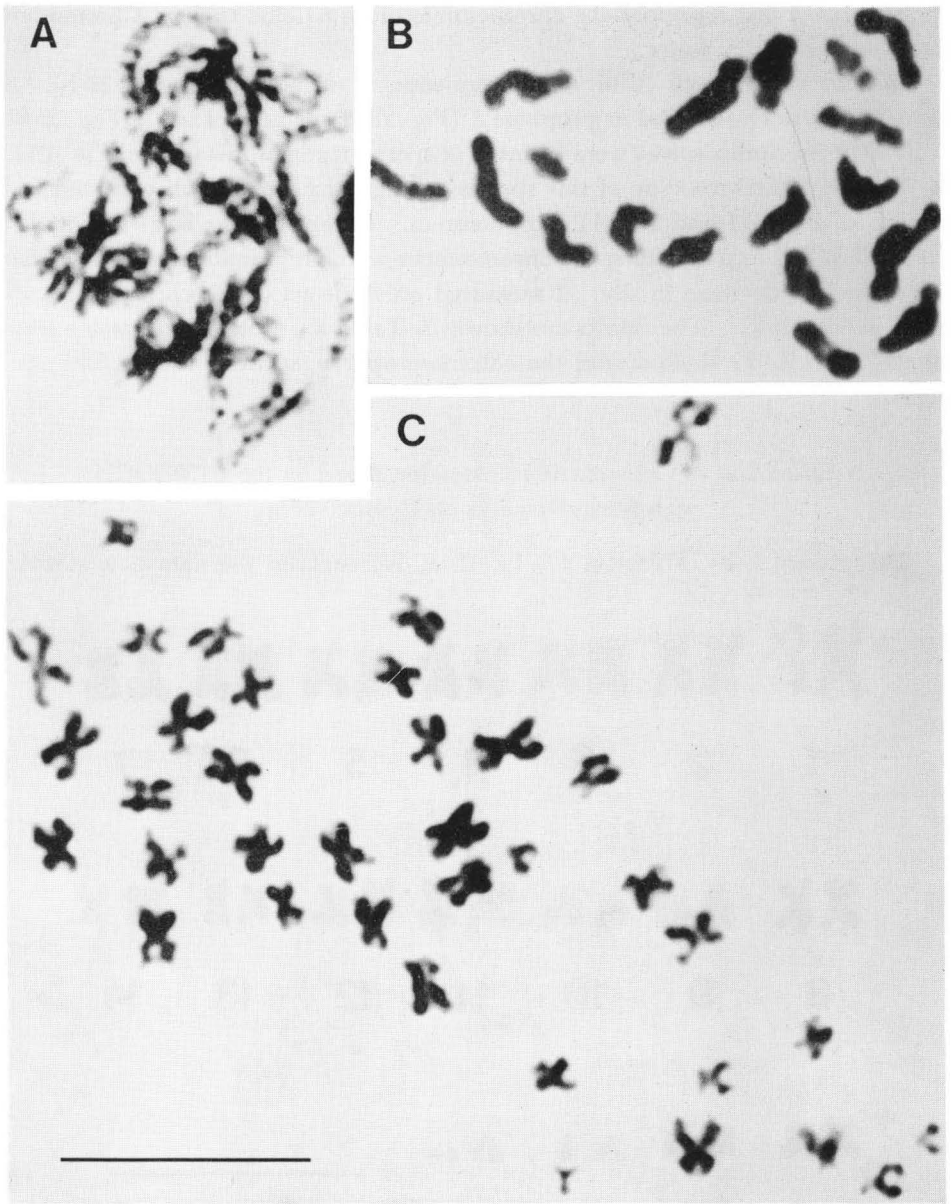

Fig. 28. Chromosomes of male *Broderipia iridescens*. A–B: Meiosis. C: Mitosis. A. Prophase I (zygotene); B. Early metaphase I (N=18); C. Spermatogonial metaphase (2N=36). Scale bar: 10 μm.

in the present study. However, Komatsu calculated the value of fundamental number as 72 instead of 68.

Stomatellidae (Trochacea)

27. *Broderipia iridescens* (Broderip, 1834)

This is the first report on the chromosomes of stomatellid snails. Chromosomes were observed in two males.

In male meiotic cells 18 bivalents were counted at metaphase (Fig. 28-B). No chromosomes were observed at prophase I (Fig. 28-A) or metaphase I (Fig. 28-B).

Thirty-six chromosomes were counted at spermatogonial metaphase (Fig. 28-C). Fig. 29 shows the karyotype of this species arranged by size, which consisted of 14 pairs of M, 2 of SM and 2 of ST chromosomes. Except for one largest M and the two smallest SM and ST pairs, the chromosomes were very similar in size and shape with a gradual decrease in size. I measured only one set of the chromosomes presented in Fig. 28-C. The results are shown in Table 8. Total chromosome length was 95.75 μm in the diploid, and the chromosomes ranged from 1.63 to 3.97 μm in length.

A Check List of Cytogenetic Information Based on the CISMOCH, with Some Remarks on Cytotaxonomy

The present list (Tables 10, 12, 14, 15 & 16) contains the data now stored in

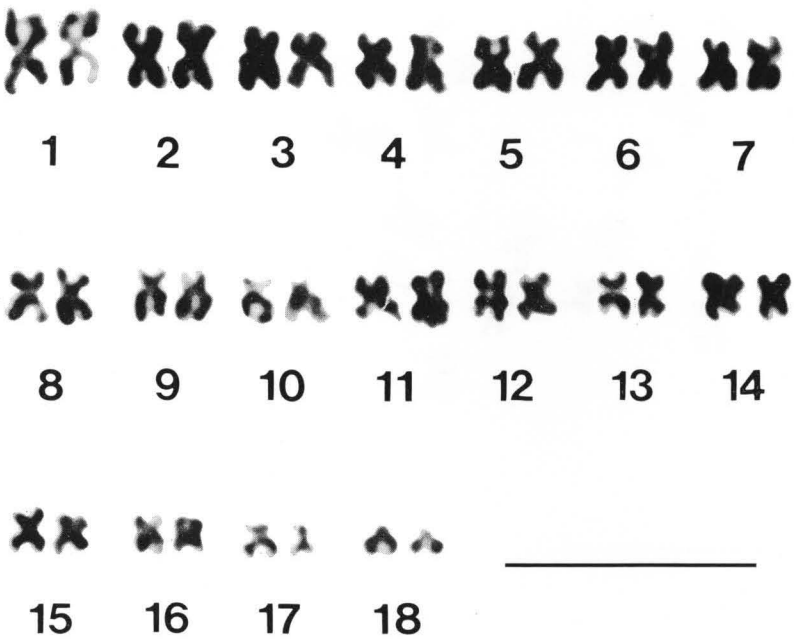

Fig. 29. Karyotype of *Broderipia iridescens* constructed from Fig. 28-C. Scale bar: 10 μm.

the CISMOCH, Computerized Index System for Molluscan Chromosomes, which provides as complete a survey as possible of the available information up to 1985, in order to facilitate collection of further data and critical review of older and incomplete information. Details of the system were presented in my recent article (Nakamura, 1985c).

Explanation of the list

A. *Order of species.*

In general an alphabetical sequence has been used. Thus the order of species under any genus is always alphabetical, and the order of genera within subfamilies or families is also alphabetical. The following taxonomical suggestions were adopted on grouping at the superfamily level: Golikov & Starobogatov (1975) for Haliotidae, and Thompson (1980) for Helicinidae. Groupings at the subfamily level are indicated only in the families Fissurellidae and Trochidae, and the order of the subfamilies is alphabetical within these families. In case where the author who originally reported some chromosome information used a synonym of the species name, the present valid name has been applied.

B. *Abbreviations and symbols used in the list.*

1) Classification column
   [ ]: synonym used by the original investigator
2) Chromosome number column
   m: male, mostly spermatogonium
   f: female, mostly oogonium
   s: somatic cell, the sex unknown
   u: sex and origin of the cell material unknown
   ( ): revised or questionable count
3) Method and karyological data column

The method of chromosome preparation is indicated by the following numbers in square brackets: 0, unknown; 1, section; 2, squash; and 3, air (including warm or flame) dry.

Table 9. Number of species examined karyologically in Archaeogastropoda.

Family	No. of species examined by the investigators other than the present author*	No. of species re-examined by the present author**	No. of species newly examined by the present author	Total no. of examined species
Acmaeidae	11 (5)	4 (4)	3	14
Patellidae	4 (3)	3	1	5
Neritidae	21 (15)	8 (2)	2	23
Haliotidae	5 (2)	1 (1)	2	7
Fissurellidae	3 (3)	2 (1)	3	6
Trochidae	10 (9)	5 (1)	4	14
Turbinidae	3 (3)	1	0	3
Stomatellidae	0 (0)	0	1	1
Helicinidae	3 (0)	0	0	3
Total	60 (40)	24 (9)	16	76

*: The number of species from Japan is given in parentheses. **: The number of species whose chromosome numbers are different from the results reported by the previous workers is given in parentheses.

Table 10. Chromosomes of Acmaeidae and Patellidae.

Classification	Chr No. 2n n	[Method] Karyological Data	Locality	Source	Note
PATELLACEA					
Acmaeidae					
Collisella digitalis	s20 m, f10	[2] Type; 4M, 5SM, 1ST FN=36*	Oreg., USA	Chapin & Roberts (1980)	
C. helordi	m20 m10	[3] Type; 5M, 1M/SM, 4SM FN=38 TCL=49.02 CL; 0.91–4.79**	Shirahama, Wakayama, JAPAN	Nakamura (1982b)	
C. langfordi	m20 m10	[3] Type; 4M, 2M/SM, 2SM, 2SM/ST FN=34–38 TCL=49.32 CL; 0.67–4.96**	Shirahama, Wakayama, JAPAN	Nakamura (1982b)	
C. pelta	s20 m, f10	[2] Type; 3M, 6SM, 1ST FN=36*	Oreg., USA	Chapin & Roberts (1980)	
C. strigatella	s20 m, f10	[2] Type; 5M, 3SM, 2ST FN=32*	Oreg., USA	Chapin & Roberts (1980)	
Notoacmea concinna	m20 m10	[3] Type; 4M, 1M/SM, 2SM, 1SM/ST, 1ST/T, 1T FN=32/34 TCL=46.69** CL; 0.83–4.06**	Shirahama, Wakayama, JAPAN	Nakamura (1982a)	
N. concinna	(m9)	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
N. fenestrata	s20 m, f10	[2] Type; 4M, 5SM, 1ST FN=38*	Oreg., USA	Chapin & Roberts (1980)	
N. fuscoviridis	m20 m10	[3] Type; 3M, 3SM, 2SM/ST, 1ST/T, 1T FN=32–36 TCL=41.93** CL; 0.61–3.71**	Shirahama, Wakayama, JAPAN	Nakamura (1982a)	
N. fuscoviridis	(m9)	[1]	Yamaguchi, JAPAN	Nishikawa (1962)	
N. persona	s20 m, f10	[2] Type; 4M, 6SM FN=38*	Oreg., USA	Chapin & Roberts (1980)	
N. schrenkii	m, f20 m10	[3] Type; 3M, 1M/SM, 2SM, 2SM/ST, 1ST, 1T FN=32/34 TCL=48.33** CL; 0.64–3.58**	Shirahama, Wakayama, JAPAN	Nakamura (1982a)	
N. schrenkii	(m9)	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
N. scutum	s20 m, f10	[2] Type; 4M, 4SM, 2ST FN=32*	Oreg., USA	Chapin & Roberts (1980)	
Patelloida pygmaea lampanicola	(m9)	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	

P. P. pygmaea		(m9)	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
P. P. pygmaea		m10	[2]	Kochi Pref., JAPAN	Colombera (pers. comm.)	
P. saccharina lanx	m20	m10	[3] Type; 10M FN=40 TCL=52.84 CL; 1.64–4.08	Shirahama, Wakayama, JAPAN	present study	1
P. saccharina lanx		(m9)	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
P. striata	m20	m10	[3] Type; 7M, 1SM, 1ST, 1T FN=34 TCL=50.89 CL; 1.03–3.60	Toka, Okinawa Is., JAPAN	present study	2
Patellidae						
Cellana grata	m18	m9	[3] Type; 9M FN=34 TCL=47.73 CL; 1.02–3.75	Shirahama, Wakayama, JAPAN	present study	
C. grata [=*C. eucosmia*]	m18	m9	[1, 2]	Shimonoseki, JAPAN	Nishikawa (1962)	
C. nigrolineata	m18	m9	[3] Type; 9M FN=34 TCL=37.38 CL; 1.06–2.77	Shirahama, Wakayama, JAPAN	present study	
C. nigrolineata		m9	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
C. toreuma	m18	m9	[3] Type; 7M, 1M/SM, 1SM FN=34 TCL=36.17 CL; 0.88–3.24**	Toka, Okinawa Is., JAPAN	Nakamura (1982b)	
C. toreuma		m9	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
Patella flexuosa	m18	m9	[3] Type; 8M, 1T FN=34 TCL=57.41 1.22–4.66	Shirahama, Wakayama, JAPAN	present study	
P. vulgata	m18	m9	[2] Type; 6M/SM, 3T* FN=30* TCL=@48* CL; 1.5–4.6*	Gulf of Palermo, ITALY	Vitturi et al. (1982)	

Notes: 1. No microchromosomes are observed; 2. Two pairs of the chromosomes are very small M and T. See text for further explanations.

Other karyological data are arranged and listed according to the items mentioned below:

Type: nomenclature according to Levan et al. (1964).

FN: fundamental number or "nombre fondamental" (Matthey, 1945), calculated on the basis that M and SM chromosome have a value of 4, ST and T chromosomes have a value of 2, and the microchromosomes in the Patellacea, regardless of morphology, have a value of 2 in a haploid set.

CL: length (μm) of the mitotic metaphase autosomes.

TCL: total length (μm) of the mitotic metaphase autosomes.

*: counted or measured by the present author.

**: unpublished data of the present author.

4) Comments

Any comments or data not presented in the list are mentioned in the footnote. Each comment is indicated by the number in the Note column. The asterisk indicates that the comment is made by the present author, and the others by the original investigator(s).

Cytotaxonomical remarks

Chromosomal information on a total of 76 archaeogastropod species from nine families is now available (Table 9). Most of the early results are from Nishikawa (1962). He worked on chromosomes of 25 species of the Archaeogastropoda, most of which were done on first meiotic metaphase using the classical sectioning method.

1. Acmaeidae and Patellidae (Patellacea)

Patellacea includes a total of about 200 species in three families. Excepting the smallest family, Lepetidae, there is reliable chromosome information for 19 species (Table 10). According to the results of Nishikawa's earlier work (Nishikawa, 1962), all the species were thought to have the same chromosome number, N=9, both in Acmaeidae and Patellidae. Recently, however, it has been revealed that the Acmaeidae have the chromosome number, N=10. No variation in chromosome number has been reported in either family.

In all species of the genera *Collisella, Notoacmea* (Acmaeidae) and *Cellana* (Patellidae) the karyotype is quite peculiar. They have an extremely small chromosome pair (No. 10 in the Acmaeidae and No. 9 in the Patellidae), which is clearly distinct in size (see Idiograms in Figs. 30 & 31). These smallest chromosomes vary from one-half to two-thirds of the second smallest chromosome pair in length, and are around 1 μm long (Table 11). They are not so small as the so-called B-chromosomes in the Aves but are clearly distinguishable by small size and lighter color in both meiotic and mitotic metaphase from the others. The rest of the chromosomes are all M in *Cellana* and one or two SM/ST are usually found in *Collisella* and *Notoacmea*.

Exceptions to the basic karyotypic characteristics mentioned above have been encountered in the species of *Patelloida* (Acmaeidae) and *Patella* (Patellidae). *Patelloida saccharina lanx* lacks that kind of remarkably small chromosomes in the karyotype. The chromosomes of this species are all M gradually decreasing in size with no apparent size distinction appearing (Fig. 30). The smallest chromosome in *Patelloida striata* is about 1 μm long and resembles the one in other acmaeids. In

Chromosome measurements are shown in Table 8.

Komatsu (1984) reported chromosome number and centromere positions of this species, which very much coincided with the present observation in morphology and total length as well as in number of chromosomes although the applied methods were different, i.e. aceto-orcein squash method in Komatsu's work and warm-dry method

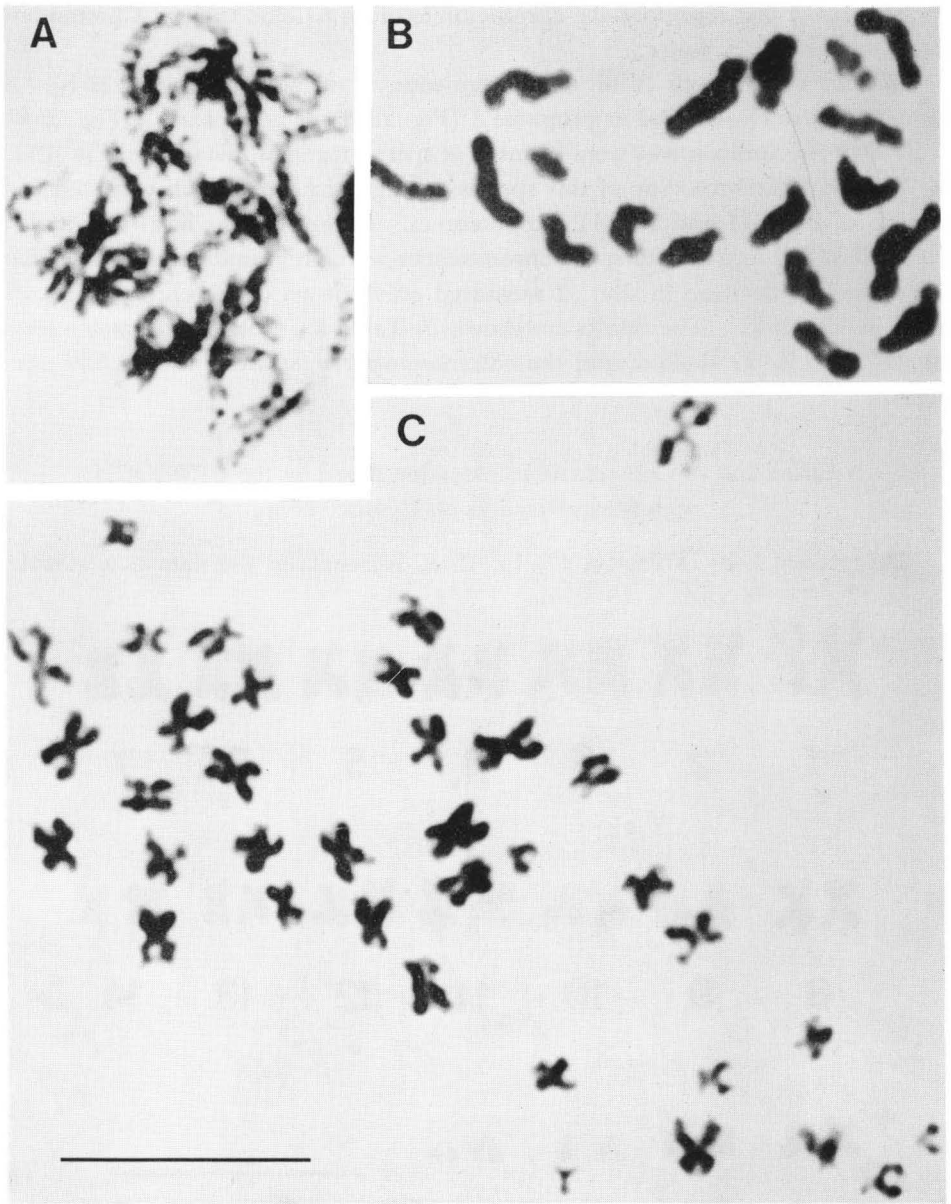

Fig. 28. Chromosomes of male *Broderipia iridescens*. A–B: Meiosis. C: Mitosis. A. Prophase I (zygotene); B. Early metaphase I (N=18); C. Spermatogonial metaphase (2N=36). Scale bar: 10 μm.

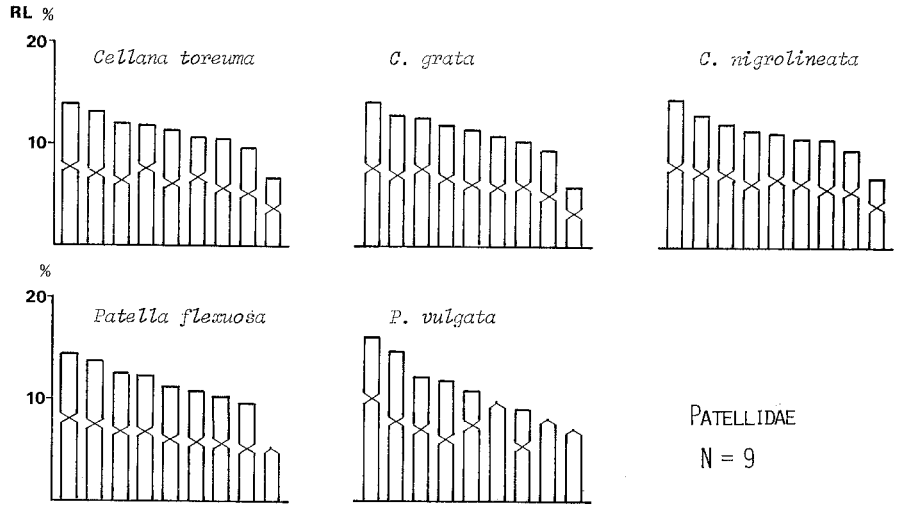

Fig. 31. Idiograms of the Patellidae (Patellacea) drawn from the data in Table 4 and Nakamura (1982b), and from the measurements by the present author on the photograph of Vitturi et al. (1982). The per cent scale is the relative length (RL).

distinguishable at mitotic metaphase as it is small, about half of the No. 8, and it is an apparent T without short arm, but its degree of staining seems to be equal to that of the others. Therefore, at first meiotic metaphase, this chromosome is rarely distinguished only by its size from the other chromosomes. In the karyotype of *Patella vulgata* the No. 9 chromosome pair is again T but it is rather larger than in the previous species. Consequently there is no apparent size distinction between the No. 9 and No. 8 chromosomes (Table 11 & Fig. 31). Moreover this species has a few T chromosomes though in the other patellids no T chromosomes have been recorded except the No. 9 of *Patella flexuosa*.

For the present, we have two groups characterized by chromosome number: N=9 corresponding to the family Patellidae and N=10, to Acmaeidae. In most species of each family the smallest chromosomes are identical and likely homologous. Based on these karyological data, however insufficient they are, at least three different views on phylogeny can be constructed. The first is that the patellids are considered primitive with the transition from patellids to acmaeids taking between *Cellana* and *Collisella-Notoacmea,* which is supported by the hypothesis concerning karyological tendency in molluscs postulated by Burch (1965) and others: the primitive molluscs have lower chromosome numbers. As the second possibility the phylogeny is reversed, with the acmaeids being considered primitive, supported by Vitturi et al. (1982) and others: evolution within Mollusca is accompanied by a decrease in chromosome number. The transition may be regarded as taking place between *Collisella-Notoacmea* and *Cellana.* The third possibility may place acmaeids and patellids as derived from a common ancestor, and the one is not a stem group of the other. Then I will review the previous hypotheses on the phylogeny of the Patellacea deduced from the morphological and anatomical studies,

Table 11. Measurements of the smallest chromosomes in Patellacea.

Species	AL	RL	PSS	Source	Shell structure group**
Acmaeidae					
Collisella digitalis	—	4.3	53.8	Chapin and Roberts, '80	—
C. heroldi	1.14	4.32	56.2	Nakamura, '82b	—
C. langfordi	0.93	3.64	44.5	Nakamura, '82b	—
C. pelta	—	4.0	50.6	Chapin and Roberts, '80	1
C. strigatella	—	4.4	57.1	Chapin and Roberts, '80	1
Notoacmea concinna	0.89	3.81	48.0	Nakamura, '82a	—
N. fenestrata	—	4.6	67.6	Chapin and Roberts, '80	1
N. fuscoviridis	0.76	3.63	43.6	Nakamura, '82a	—
N. persona	—	4.1	63.1	Chapin and Roberts, '80	1
N. schrenckii	0.84	3.48	41.0	Nakamura, '82a	—
N. scutum	—	4.8	68.6	Chapin and Roberts, '80	1
Patelloida saccharina lanx	1.93	7.30	90.0	present study	2
P. striata	1.23	4.84	84.5	present study	1
Patellidae					
Cellana grata	1.38	5.79	61.0	present study	12
C. nigrolineata	1.27	6.81	71.0	present study	12
C. toreuma	1.23	6.78	70.7	Nakamura, '82b	12
Patella flexuosa	1.52	5.31	55.3	present study	9
*P. vulgata**	1.70	7.07	86.3	Vitturi et al., '82	8

AL: actual length (μm); RL: relative length (% length in haploid); PSS: ratio of the smallest chromosome length to the second smallest chromosome length (i.e., No. 10 chromosome length÷No. 9×100 in Acmaeidae and No. 9÷No. 8×100 in Patellidae); *: measured by the present author on the photographs offered by the original author(s); **: after MacClintock (1967).

and consider the probability of above three views.

Patellacea includes three recent families. They are distinguished by the following anatomical criteria. The Acmaeidae are the only patellaceans with a ctenidium, and some species also have a secondary gill (branchial cordon). The Lepetidae lack the gills completely and the Patellidae have only a secondary gill. The evolution in this group has been drawn on the basis of gill structure or radula formula, traditionally both of which have been regarded as the most important characteristics in Gastropoda systematics. The above mentioned second possibility is supported by the widely accepted patellacean phylogeny based mainly gill structure (Yonge, 1947), imposing those with a single ctenidium as primitive and those with 'specialized' pallial gills only as advanced. On the other hand, the first possibility can be supported by the recently suggested phylogeny based on radula formula (for example Golikov & Starobogatov, 1975), with the progression from a complex to a simple radula.

MacClintock (1967) introduced a new character into patellacean systematics:

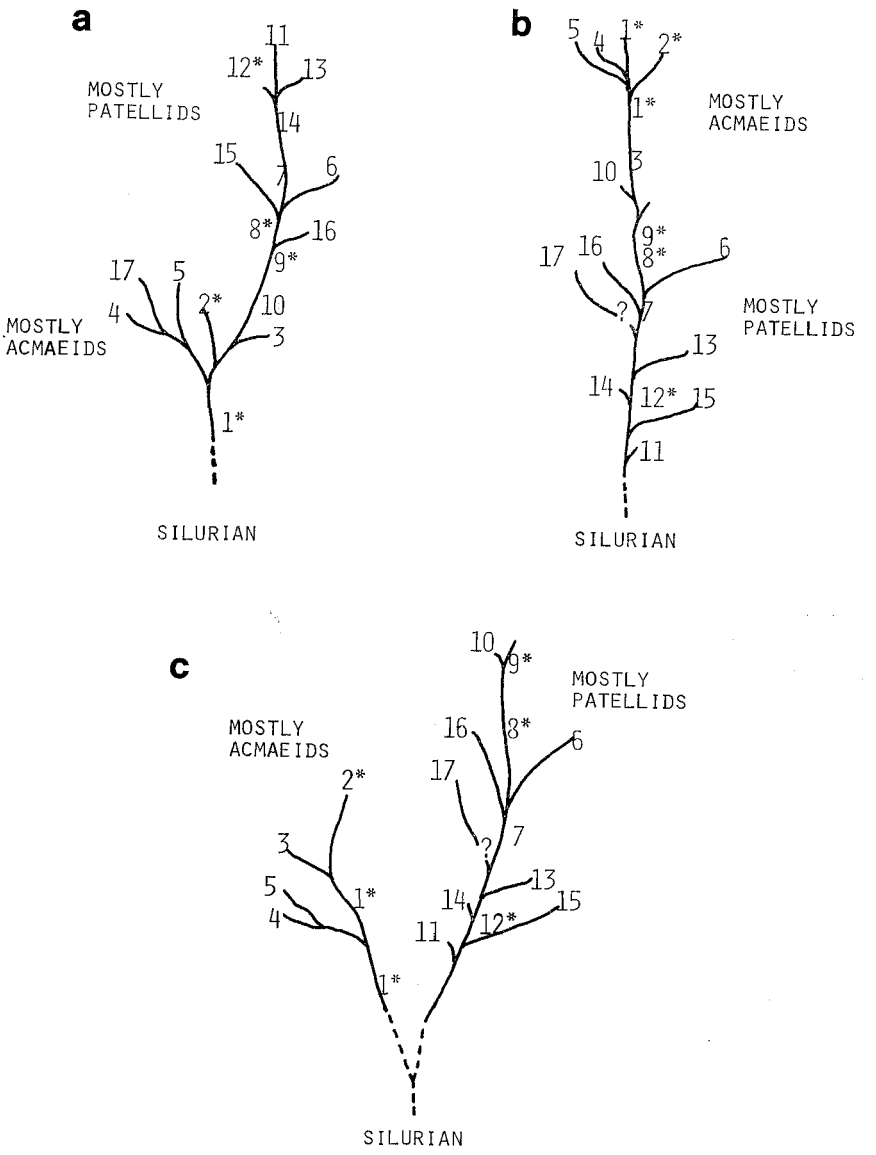

Fig. 32. Dendristic diagram showing morphologic and possibly phylogenetic relationships among the 17 patellacean shell-structure groups (after MacClintock, 1967, slightly modified). **a**, with acmaeids primitive. **b**, with patellids primitive. **c**, with acmaeids and patellids derived common ancestor. Shell-structure groups are indicated with the numbers; Group No. 17 restricted to Eocene patellaceans. The shell-structure groups indicated by asterisks are including the species studied karyologically.

shell structure. He found 17 different types of crystal structure and layering in recent and fossil patellacean shells. The shell structure of 11 species out of 19 karyologically investigated patellaceans were treated by MacClintock (Table 11). Six of seven acmaeids, except *Patelloida saccharina lanx*, belong to the Shell Structure

Group 1; *Patelloida saccharina lanx*, to Group 2; *Patella vulgata*, to Group 8; *Patella flexuosa*, Group 9; and three cellanids, to Group 12.

Among 17 groups there is nearly a complete gradation of structural types, from which three possible phylogenies were deduced (Fig. 32) in the same way as implied from karyotypes. Supposing that the unique smallest chromosomes in *Cellana* and most acmaeids are karyologically homologous, their karyotypes are thought to be closely related to each other. Therefore, among the three phylogenies constructed by MacClintock based on shell structure, the phylogeny with acmaeids and patellids derived from a common ancestor (Fig. 32**c**) seldom incurs contradiction; for in the other two possible phylogenies, *Cellana* (Group 12) and most acmaeids (Group 1) are rather distant.

This phylogeny assumes that within the superfamily Patellacea, the radula and gill structure have changed independently: during the evolution of the family Patellidae the radula apparatus has remained 'primitive' with numerous teeth, whereas the 'primitive' ctenidium has been left in the Acmaeidae. Of course this is a tentative suggestion because it is yet to be determined whether the unique smallest chromosomes in *Cellana* and most acmaeids are homologous, and whether there is any overall association between karyotypic characters and shell structure.

Lindberg (1981) suggested that the Rodopetalinae, with the combination of the patellid shell structure (Group 12) and acmaeid-like anatomy, would be an ancestral intermediate group between the acmaeids and cellanids limpets. If both the above assumed phylogeny and Lindberg's suggestion are true, then the Rodopetalinae can also be expected to have the unique smallest chromosome in the karyotype as in *Collisella, Notoacmea* and *Cellana*.

2. Neritidae (Neritacea) and Helicinidae (Helicinacea)

The Neritidae live mostly along shore lines in marine and brackish water and many found in fresh water as well. This family comprises nearly 200 species, mostly tropical and subtropical areas. Twenty-three species from Europe, India, Hong Kong and Japan have been karyologically investigated for this family (Table 12).

In all marine species of this family karyotype is quite similar. They have a small chromosome number of N=12, 2N=23 (♂) and 2N=24 (♀) with one pair of large M (R.L. larger than 13%) chromosomes. The value of FN of autosomes varies from 40 to 44 (average 43), i.e., ST/T chromosomes seldom appear in their karyotypes. An allocyclic heterochromatic chromosome is found in male meiosis in all species and an XO–XX sex-determining mechanism is postulated.

Exceptions to this basic karyotype have been encountered in fresh and brackish water species. *Clithon oualaniensis* has the same chromosome number of N=12 as the marine species, but the morphologies of its complements are very different (Komatsu, 1983). There are five pairs of T chromosomes and thus the value of FN is very small, FN=34. *Neritina pulligera* also has a chromosome number of N=12. However, its FN is slightly smaller, FN=40, and the total autosome length is rather shorter than in the marine species, according to my measurements on the

Table 12. Chromosomes of Neritidae and Helicinidae.

Classification	Chr 2n	No. n	[Method] Karyological Data	Locality	Source	Note
NERITACEA						
Neritidae						
Clithon corona		m12	[2]	Ishigaki Is., Okinawa, JAPAN	Komatsu & Inaba (1982)	
C. oualaniensis	m23; 22+x f24; 22+xx	m12; 11+h	[2] Type; 3M, 3SM, 5T+x(M) FN=34	JAPAN	Komatsu (1983)	
C. oualaniensis [=*Neritina oualaniensis*]	m23; 22+x	m12; 11+x	[0]	Andaman Is. & South India, INDIA	Natarajan (1969)	
C. retropictus		m12	[2]	Hiroshima Pref., JAPAN	Komatsu & Inaba (1982)	
C. retropictus		u12; 11+h (male?)	[2]	Shimoda, Shizuoka, JAPAN	Patterson (1967)	
Nerita albicila	m23; 22+x f24; 22+xx	m12; 11+h	[3] Type; 8M, 1M/SM, 2SM+x(T) FN=44 TCL=60.65 CL; 1.36–6.79	Shirahama, Wakayama, JAPAN	present study	
N. albicila		m12; 11+h	[2]	Tokushima Pref., JAPAN	Komatsu & Inaba (1982)	
N. dombeyi		m12; 11+x	[0]	Andaman Is., INDIA	Natarajan (1969)	
N. helicinoides laevilabris		m12; 11+h	[3]	Gizabanta, Okinawa Is., JAPAN	present study	
N. h. laevilabris		m12; 11+h	[2, 3]	Sesoko Is., Okinawa, JAPAN	Komatsu & Inaba (1982)	
N. incerta		m12; 11+h	[2]	Okinawa, JAPAN	Komatsu & Inaba (1982)	
N. insculpta	m23		[3]	Gizabanta, Okinawa Is., JAPAN	present study	
N. insculpta		m12; 11+h	[2]	Okinawa, JAPAN	Komatsu & Inaba (1982)	
N. japonica	m23; 22+x f24; 22+xx	m12; 11+h	[3] Type; 9M, 1M/SM, 1SM+x(SM) FN=44 TCL=61.56 CL; 1.31–5.80	Shirahama, Wakayama, JAPAN	present study	
N. japonica [=*Puperita (Heminerita) japonica*]	m23; 22+x		[2, 3] Type; 9M, 2SM+x(SM) FN=44* TCL=@75*	Hiroshima Pref., JAPAN	Komatsu & Inaba (1982)	
N. japonica [=*P. (H.) japonica*]	(m22)	(m11)	[1, 2]	Shimonoseki, JAPAN	Nishikawa (1962)	
N. lineata	m23	m12; 11+h	[3]	Hong Kong	Nakamura (1985a)	
N. ocellata	m23; 22+x	m12; 11+h	[2, 3] Type; 9M, 2SM+x(SM) FN=44* TCL=39*	Okinawa Is., JAPAN	Komatsu & Inaba (1982)	
N. plicata	m23	m12; 11+h	[3]	Gizabanta, Okinawa Is., JAPAN	present study	

N. plicata		m12; 11+h	[2]	Sesoko Is., Okinawa, JAPAN	Komatsu & Inaba (1982)
N. plicata	m23; 22+x	m12; 11+x	[0]	Andaman Is., INDIA	Natarajan (1969)
N. polita	m23; 22+x f24	m12; 11+h	[3] Type: 6M, 3SM, 1ST, 1T+x(SM) FN=40 TCL=61.91** CL; 1.45–4.97**	Hong Kong	Nakamura (1985a)
N. polita		m12; 11+h	[2, 3]	Sesoko Is., Okinawa, JAPAN	Komatsu & Inaba (1982)
N. polita [=*N. rumphii*]		m12; 11+x	[0]	Andaman Is., INDIA	Natarajan (1969)
N. squamulata		m12; 11+h	[2]	Okinawa Is., JAPAN	Komatsu & Inaba (1982)
N. squamulata	m23; 22+x	m12; 11+h	[3] Type; 9M, 1SM, 1ST+x(SM) FN=42 TCL=38.56** CL; 1.47–2.97**	Hong Kong	Nakamura (1985a)
N. squamulata [=*N. chamaeleon*]		m12; 11+x	[0]	Andaman Is., INDIA	Natarajan (1969)
N. striata	m23; 22+x	m12; 11+h	[2, 3] Type; 8M, 3SM+x(SM) FN=44* TCL=@49*	Ishigaki Is. & Okinawa Is, JAPAN	Komatsu & Inaba (1982)
N. yoldii	m23; 22+x f24; 22+xx	m12; 11+h	[3]	Hong Kong	Nakamura (1985a)
Neritina auriculata		m12; 11+x	[0]	South India, INDIA	Natarajan (1969)
N. pulligera	m23; 22+x f24; 22+xx	m12	[2] Type; 7M, 2SM, 2ST+x/xx(SM) FN=40* TCL=@43*	Ishigaki Is., JAPAN	Komatsu & Inaba (1982)
N. retifera	m23; 22+x	m12; 11+x	[0]	South India, INDIA	Natarajan (1969)
N. violacea	m27; 26+x	m14; 13+h	[2] Type; 6M, 3SM, 4T+x(SM) FN=44* TCL=@42	Fukuoka Pref., JAPAN	Komatsu & Inaba (1982)
N. violacea	m23; 22+x	m12; 11+x	[0]	South India, INDIA	Natarajan (1969)
N. violacea [=*Dostia violacea*]		u14;13+h (male?)	[2]	Yatsushiro, Kumamoto, JAPAN	Patterson (1967)
N. violacea [=*D. violacea*]	m21; 20+x f22; 20+xx	m11; 10+h	[3] Type; 6M, 2SM, 1T, 1?+x(SM) FN=36/38 TCL=44.25** CL; 1.25–4.65**	Hong Kong	Nakamura (1985a)
Septaria compressa		m12; 11+x	[0]	South India, INDIA	Natarajan (1969)
S. tessellata	m23; 22+x f24; 22+xx	m, f12; 11+x	[0]	South India, INDIA	Natarajan (1969)
Theodoxus fluviatilis [=*Nerita fluviatilis*]	m19; 18+x f20; 18+xx	f10	[1]	Kiyev, USSR	Alexenko (1928)
T. fluviatilis [=*Theodoxia fluviatilis*]	m18; 16+xy	m9; 8+x/y	[1]	Montpellier, FRANCE	Tuzet (1930)
HELICINACEA					
Helicinidae					
Palaehelicina sp.		u18	[0]	Solomon Is.	Burch (1967)
Pleuropoma sp. 1		u18	[0]	Solomon Is.	Burch (1967)
Pleuropoma sp. 2		u18	[0]	Solomon Is.	Burch (1967)

See text for explanations.

photograph offered by Komatsu & Inaba (1982). Finally, intraspecific polymorphism in chromosome number was found in the European brackish water species *Theodoxus fluviatilis* (N=9 and 10), and the Asian *Neritina violacea* (N=11, 12 and 14). The neritids are the only group to expand their habitat from the sea to brackish and fresh water in the Archaeogastropoda, then the above-mentioned karyotypical instability in brackish and fresh water species could be related to their obtaining new niches.

The comparison of the karyotypes in the intraspecifically polymorphic species of *Neritina violacea* among local groups from Japan (N=14), Hong Kong (N=11) and India (N=12) suggests the mechanism of chromosome alternation. Assuming that non-marine neritids evolved from marine ancestry, the karyotype of the Japanese *N. violacea* (N=14) can be attributed to Robertsonian fission because it has the fundamental number, FN=44, which corresponds to that of most marine neritids. On the other hand, Hong Kong *N. violacea* (N=11) has FN=36/38 and therefore the Robertsonian translocation cannot explain the mechanism of karyotype evolution in Hong Kong *N. violacea*.

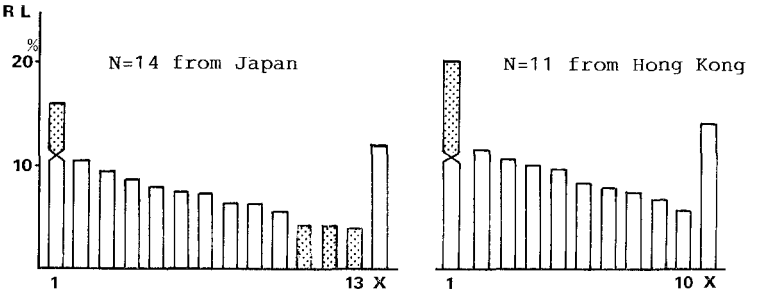

Fig. 33. Idiograms of *Neritina violacea* N=14 (from Japan) and N=11 (from Hong Kong) populations, drawn from the measurements on the photographs in Komatsu & Inaba (1982) and Nakamura (1985a). Combining the smallest three chromosomes and the largest chromosome of N=14 population could explain the difference of chromosome number between these two 'races' (tandem fusion). The centromeric position is shown only on the largest chromosome. The per cent scale is the RL. X: sex chromosome.

There are no remarkable differences in the total autosome length and in shape and size of the sex chromosome between two populations of *N. violacea* from Japan and Hong Kong. On the other hand, as seen in Fig. 33, in the karyotype of the Hong Kong population, the largest No. 1 chromosome is even larger and is metacentrically constructed instead of being submetacentric as in the No. 1 chromosome of the Japanese N=14 population, while the karyotype of the Hong Kong population lacks the chromosomes corresponding to the three smallest chromosomes in the karyotype of the Japanese population, Nos. 11, 12 and 13 pairs. This may lead to a hypothesis that the three smallest pairs of N=14 population were tandemly translocated to the largest No. 1 submetacentric pair to form the largest No. 1 metacentric pair of N=11 population. Admitting this hypothesis, that is, in *N. violacea* the chromosome number decreased with tandem fusions from N=14 to N=11,

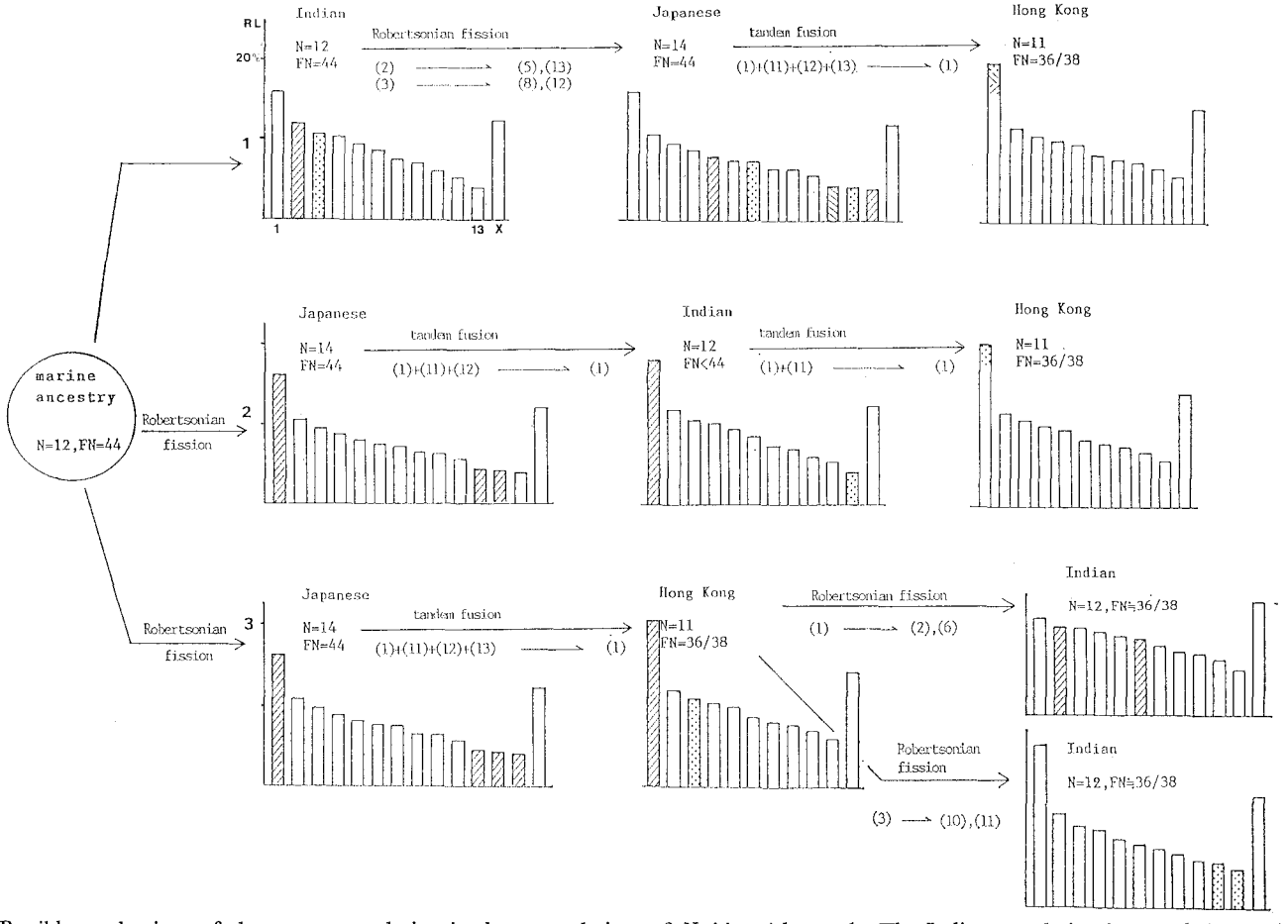

Fig. 34. Possible mechanisms of chromosome evolution in three populations of *Neritina violacea*. 1. The Indian population is regarded as primitive; 2. The Indian population is regarded as intermediate in the evolutionary course from N=14 to N=11: chromosome number changing with tandem fusion; 3. The Indian population is regarded as advanced: The figures show two possible results, (top) No. 1 chromosome (Hong Kong population) splitting to be No. 2 and No. 6 chromosomes (Indian population), or (bottom) No. 3 chromosome (Hong Kong population) splitting to be No. 10 and No. 11 chromosomes (Indian population). The per cent scale is the RL. X: sex chromosomes.

phylogenetic status of Indian N=12 *N. violacea* in the lineage can be supposed karyologically as follows (Fig. 34). The Indian population has (1) an identical karyotype to the marine neritids with FN=44 as an ancestor of the N=14 group (Komatsu & Inaba, 1982), (2) one small chromosome pair in the karyotype corresponding to either the No. 11, 12 or 13 chromosome in the karyotype of the Japanese *N. violacea* as an intermediate group in the evolutionary course from N=14 to N=11, or (3) two chromosome pairs originated from one pair of the chromosomes in the karyotype of the Hong Kong *N. violacea* as the most specialized group in this lineage. The second and third cases mean that though the Indian *N. violacea* has the same chromosome number, N=12, as the marine neritids, phylogenetically it is not closer to the marine ancestry than the N=14 group of *N. violacea*. Unfortunately as no mitotic figures or other details were published on the Indian population, these are left for future investigation.

Difference in chromosome number in a single species was also reported from a European brackish water neritid, *Theodoxus fluviatilis* (N=9: Tuzet, 1930; and N=10: Alexenko, 1928). These reports were based on the classical sectioning method, and thus the results have been doubted (Nishikawa, 1962). This species, however, may have intraspecific polymorphism as shown in *Neritina violacea*, and reexamination using modern cytological techniques is very much anticipated.

Sex (X) and heterochromatic (h) chromosomes have been found or postulated to exist by all previous workers studying neritid chromosomes except Nishikawa (1962). Nishikawa reported 11 haploid chromosomes in metaphase I and II, and 22 chromosomes in spermatogonia of male *Nerita japonica* without finding any special chromosomes. But this species, reexamined by Komatsu & Inaba (1982) and in the present study, was revealed to have N=12 including one h-chromosome in male meiotic metaphase I, and 2N=23 including one non-paired X-chromosome

Table 13. Sex chromosomes in Neritidae.

Species	PST	AL	Type	Rank	Source
Nerita albicila	7.56	2.29	T	8	present study
N. japonica	13.97*	5.14*	SM	1	Komatsu and Inaba, '82
N. japonica	14.08	4.54	SM(2.14)	1	present study
N. ocellata	13.08*	2.55*	SM	3	Komatsu and Inaba, '82
N. polita	14.25	4.41**	SM(1.88)	2	Nakamura, '85a
N. squamulata	13.43	2.58**	M(1.41)	2	Nakamura, '85a
N. striata	10.68*	2.64*	SM	4	Komatsu and Inaba, '82
Neritina pulligera	15.14*	3.26*	SM	2	Komatsu and Inaba, '82
N. violacea	14.52*	3.27*	SM	2	Komatsu and Inaba, '82
N. violacea	14.29*	3.16**	SM(1.86)	2	Nakamura, '85a

PST: ratio of the sex chromosome length to the total haploid autosome length in percentage; AL: actual length (μm) of the sex chromosome; Type: nomenclature after Levan, et al. (1964) Rank: size rank of the sex chromosome in decreasing order of the haploid chromosome complement; *: calculated/measured by the present author; **: unpublished data of the present author.

in males and 2N=24 including one pair of X-chromosomes in females. Therefore the Neritidae can be characterized as having the sex-determining mechanism of the XX–XO type, that is, XX in females and XO in males.

The sex chromosome appears allocyclic in male meiosis (Nakamura, 1985a; and the present study): it reaches maximum condensation at some stage other than late metaphase I. This chromosome shows differential and then heterochromatic staining. Quantitative data on the X-chromosomes at mitotic metaphase have been reported for eight species (Table 13). All the X-chromosomes except the one in *Nerita albicila* seem to be very similar in size and shape. They are SM or some times M and are one of the largest chromosomes in the diploid. The actual lengths are shorter in *N. ocellata, N. squamulata* and *N. striata,* which seems to be due to the over-condensation of the chromosomes as seen by their small TCL. The X-chromosome in *N. albicila* is distinctive in the mitotic metaphase; it is smaller and lacks short arms, a T chromosome, although it shows identical characters in male meiosis. As the sex chromosomes in brackish species of *Neritina pulligera* and *N. violacea* seem identical to those of marine species, changes in morphology in the sex chromosomes may be independent of their acquiring new niche.

Chromosome studies of the Helicinidae, the terrestrial group of the Archaeogastropoda, are very scarce; those few species that have been investigated are listed in Table 12, all of which were reported by Burch (1967) from the Solomon Islands. As no figures of chromosomes or other details were found in his report, there is no karyological information on this unique group except that they have an identical chromosome number of N=18.

The helicinid families were generally assigned to the superfamily Neritacea and regarded as advanced neritids. Thus, because the chromosome number in the Helicinidae is larger than in the Neritidae, some may think that there is a tendency among the Neritacea that the more specialized group has a larger chromosome number. But recently the helicinid lineage has been evaluated to superfamily rank by Thompson (1980). He mentioned, "Which group of marine molluscs was ancestral to the Helicinacea is not clear. However, it is apparent that on the basis of the shell, the opeculum, the gill, the radula (Baker, 1923), the heart and the reproductive system (Fretter & Graham, 1962; Bourne, 1908) the Neritacea is not ancestral to the Helicinacea". Chromosomal data indicate simply that these groups are quite different in chromosome number.

3. Haliotidae (Haliotacea) and Fissurellidae (Fissurellacea)

The two Japanese abalone species investigated in the present study bring the total number of species of the family Haliotidae on which there is reliable chromosome information to seven (and one subspecies) out of fewer than 50 recent species from Japan, Italy and west America (Table 14). Three different chromosome numbers are reported in this family, i.e. N=14, 16 and 18.

Japanese abalones are reported to have N=16 from *H. aquatilis* (Nakamura, 1985b) and *H. varia,* and N=18 from *H. discus* (Arai et al., 1982) and *H. gigantea*

which have such large adult body size as assigned by Habe & Kosuge (1964) to the newly errected 'genus' *Nordotis* characterized merely by the large physical volume. Although the chromosome number is the same, the karyotype of *H. aquatilis* and *H. varia* is different: the karyotype of the latter does not have any apparent ST pairs as found in that of the former species, but has more M pairs. In contrast, an American abalone, *H. cracherodii* (Minkler, 1977)*, and Japanese members of *H. discus* (*H. d. discus, H. d. hannai* and their hybrids) are identical not only in chromosome number but in chromosome size and morphology though they are geographically very widely separated, and *H. cracherodii* is not assigned to *Nordotis*. Another chromosome number of N=14 is reported from two Italian species (Colombera & Tagliaferri, 1983), whose karyotypes are not presented unfortunately.

Abalones are very similar in anatomical features, shell shape and structure, and thus the subgeneric or generic limits have not been satisfactorily solved though the species of the family Haliotidae are now fairly well understood —the problems of the assignments of all abalones to the genus *Haliotis* are discussed by McLean (1966). In contrast to their overall similarities of morphological features, they show remarkable interspecific variability in the chromosome number as mentioned above. It is possible that their chromosome numbers play some role in drawing subdividing limits within the *Haliotis* group.

Information about the chromosomes of key hole limpets, the Fissurellidae, is available for only six species out of about 200 living species: one species from Hong Kong and five species from Japan (Table 15). The chromosome number and fundamental number are variable in this family: N=13, 14, 15 and 16, and FN =44 to 62. The chromosomes in a karyotype show a wide range in size; the smallest chromosome is less than one-half or one-third of the largest one in size except *Macroschisma dilatata*.

Previously I supposed that a change in chromosome number within this family was produced as a result of Robertsonian translocation in relation to species differentiation (Nakamura, 1983), but at that time details of the chromosome morphology were known for only one species. In the present study, I report morphologies of the chromosomes of four more species, and that the values of FN in the Fissurellidae are as variable as the chromosome number, which belied my expectation. For if Robertsonian translocation resulted in a variation in chromosome number in this family, the value of FN of each species ought to be constant or to vary only slightly (Matthey, 1973).

A considerable diversity of shell structure occurs within the family. However, on the basis of radular characteristics there seem to be at least two major groups in this family (McLean, 1984); the family Emarginulinae, *sensu* Thiele (1929), and

*As the chromosome number of *H. cracherodii*, N=18, is very different from that of two Italian species, N=14, and Minkler evaluated the number based on digestive gland cells, Colombera and Tagliaferri (1983) doubted Minkler's counting. However, two other species and one subspecies are now known to have also N=18 (Arai et al., 1982 —unfortunately Colombera and Tagliaferri did not know their results; and the present study). Judging from the photographs and descriptions offered by Minkler, I think there is no question about the accuracy of Minkler's results.

Table 14. Chromosomes of Haliotidae.

Classification	Chr No. 2n	n	[Method] Karyological Data	Locality	Source	Note
HALIOTACEA						
Haliotidae						
Haliotis aquatilis [=*H. diversicolor aquatilis*]	m32	m16	[3] Type; 8M, 5SM, 2SM/ST, 1ST FN=58–62 TCL=76.1 CL; 1.45–3.44	Shirahama, Wakayama, JAPAN	Nakamura (1985b)	
H. aquatilis [=*H.* (*Sulculus*) *japonica*]	(m34)	(m17)	[1, 2]	Shimonoseki, JAPAN	Nishikawa (1962)	
H. cracherodii	m, f36		[3] Type; 8M, 8SM, 2ST FN=68* TCL=@101* CL; @2–4*	Calif., U.S.A.	Minkler (1977)	
H. discus discus	s36		[3] Type; 10M, 8SM FN=72* [Nos. 14 & 15 CHR seem ST then FN=68.]	Shizuoka Pref. (artificial breeding), JAPAN	Arai et al. (1982)	1
H. d. hannai	s36		[3] Type; 10M, 8SM FN=72* TCL=@113* CL; @2.4–4.3* [Nos. 14 & 15 seem ST then FN=68.]	many places from Japan	Arai et al. (1982)	2
H. gigantea		m18	[3]	Tateyama, Chiba, JAPAN	present study	
H. lamellosa		m14	[2]	Gulf of Naples, ITALY	Colombera & Tagliaferri (1983)	
H. tuberculata [=*H. tubercolata*]	m28	m14	[2] CL; 2.2–4.8	Roscoff, FRANCE	Colombera & Tagliaferri (1983)	
H. varia	m32	m16	[3] Type; 13M, 2SM, 1SM/ST FN=62/64 TCL=65.39 CL; 1.41–3.63	Shirahama, Wakayama, JAPAN	present study	

Notes: 1. No apparent difference was seen in morphological characteristics of the chromosomes between the hybrids (from *H. d.* and *H. d. hannai*) and their parential varieties;
2. Despite of the remarkable distinction in electrophoretic patterns between *H. d.* and *H. d. hannai*, no difference was seen on their karyotypes. See text for further explanations.

Table 15. Chromosomes of Fissurellidae.

Classification*	Chr No. 2n n	[Method] Karyological Data	Locality	Source	Note
FISSURELLACA					
Fissurellidae					
d *Diodora quadriradiatus*	m32	[3] Type; 3M, 2M/SM, 6SM, 4SM/ST, 1ST/T FN=54–62 TCL=83.74 CL; 1.37–4.82	Hoi Sing Wan, Hong Kong	present study	
e *Tugali decussata*	f30 m, f15	[3] Type; 10M, 5SM FN=60 TCL=68.47 CL; 1.44–3.5	Shirahama, Wakayama, JAPAN	present study	
f *Macroschisma dilatata*	m32 m16	[3] Type; 10M, 4M/SM, 1SM, 1ST FN=62 TCL=64.21 CL; 1.11–2.78	Shirahama, Wakayama, JAPAN	present study	
f *M. dilatata*	m32 m16	[1, 2]	Shimonoseki, JAPAN	Nishikawa (1962)	
f *M. sinense* [=*M. sinensis*]	m16	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
h *Montfortula pulchra picta*	m28 m14	[3] Type; 10M, 4SM FN=56 TCL=58.36 CL; 1.25–3.50	Shirahama, Wakayama, JAPAN	present study	
h *M. p. picta* [=*Clypidina picta*]	(m17)	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
s *Scutus sinensis*	m26 m13	[3] Type; 7M, 2SM, 4T FN=44 TCL=58.2 CL; 1.04–5.05**	Shirahama, Wakayama, JAPAN	Nakamura (1983)	

* superfamily names indicated by an abbreviation of small letter before the species name: d, Diodorinae; e, Emarginulinae; f, Fissurellinae; h, Hemitominae; and s, Sucutinae.
See text for further explanations.

Fissurellinae. The Fissurellinae is thought to be a more recently derived group. Slight karyological difference is found between the chromosomes of the Fissurellinae, represented only by the species of genus *Macroschisma*, and the others: *Macroschisma* species have a larger chromosome number (N=16) and the chromosomes of *M. dilatata* have a small size-range; the largest chromosome in this species are not as large as those in other species (see Table 6 and Nakamura, 1983). *Diodora quadriradiatus* and *M. dilatata* have the same chromosome number of N=16, whereas their chromosome morphologies are distinct. The ratio of M chromosomes in the karyotype of the former species is very low and most chromosomes are heterobranchial, namely SM, ST and T; on the other hand the latter does not have ST or T chromosomes and the majority are M chromosomes.

There is little agreement in the literature over the arrangement of subfamilies within the Fissurellidae, especially subdivision of the 'Emarginulinae'. A number of other taxa have been proposed as subfamilies from the 'Emarginulinae': Diodorinae by Wentz (1938); Hematominae by Kuroda et al. (1971) or Golikov & Starobogatov (1975); and Scutinae by Christiaens (1973). Previously I implied that a different chromosome number might represent each subfamily (Nakamura, 1983). On this basis it is shown in the present study that *Scutus sinensis* belongs to the subfamily Scutinae (Christiaens, 1973), N=13; *Montfortula pulchra picta* to the Hemitominae (Kuroda et al., 1971), N=14; *Tugali decussata* to the Emarginulinae *sensu stricto*, N=15; and *Diodora quadriradiatus* to the Diodorinae (Wentz, 1938), N=16. Because only one representative species have been investigated in each 'subfamily', it is too early to conclude whether the difference in chromosome number is species-specific or reflects higher groupings within this family. It should be pointed out again that subfamily assignments in the Fissurellidae are at present very tenuous, for example McLean (1984) suggests that these taxa proposed as subfamilies should be treated as rather tribe level within the 'Emarginulinae'. When a thorough study is completed and karyological information is accumulated on more species, it is likely that the various species of the 'Emarginulinae' fall into some separate anatomical groups with good correspondence to the groups ranked by their chromosome numbers.

Nishikawa (1962) mentioned, based unfortunately on some miscounted chromosome numbers, "the Haliotidae and Fissurellidae to have been derived from a common ancestral type seemingly characterized by N=18" with Robertsonian fission. I cannot deny the possibility of their derivation from an ancestor with a larger chromosome number as mentioned later, but detailed morphological analysis in their chromosomes cannot prove his assumption. No relationship seems to exist between the diploid chromosome number and the fundamental number within a family or between the two families. Even if Robertsonian translocation led to their diversification in chromosome number, several changes in karyotype, i.e., pericentric inversion, hyper/hypo-diploidy etc., may have consequently perhaps primarily occurred. At any rate karyological variability merits much attention, and further accumulation of karyological information is very much required.

Table 16. Chromosomes of Trochidae, Stomatellidae and Turbinidae.

Classification*	Chr 2n	No. n	[Method] Karyological Data	Locality	Source	Note
TROCHACEA						
Trochidae						
c *Cantharidus callichroa*	m36	m18	[1, 2]	Shimonoseki, JAPAN	Nishikawa (1962)	
c *C. japonicus* [= *Thalotia japonica, T. japonicus*]		m18	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
g *Gibbula richardi*		m18	[2]	Gulf of Palermo, ITALY	Vitturi et al. (1982)	
ma *Granata lyrata*	m40?	m20?	[3]	Shirahama, Wakayama, JAPAN	present study	
ma *G. lyrata* [=*Stomatella lyrata*]		m21	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
Monodonta australis	m36		[3] Type; 15M/SM, 3ST FN=66	Chichi-jima, Bonin Is., JAPAN	present study	
mo *M. labio confusa*	m36	m18	[3] Type; 7M, 3M/SM, 3SM, 3SM/ST, 2ST FN=62–68 TCL=84.66 CL; 1.47–3.72	Shirahama, Wakayama, JAPAN	present study	
mo *M. l. confusa*		m18	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
mo *M. neritoides*	m36	m18	[3] Type; 10M, 4M/SM, 1SM, 3ST FN=66 TCL=80.46 CL; 1.30–3.62	Shirahama, Wakayama, JAPAN	present study	
mo *M. neritoides*		m18	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
mo *M. perplexa*	m36	m18	[3]	Shirahama, Wakayama, JAPAN	present study	
mo *Pictodiloma suavis*	m36	m18	[3]	Shirahama, Wakayama, JAPAN	present study	
t *Chlorostoma argyrostoma lischkei*	m36	m18	[3]	Shirahama, Wakayama, JAPAN	present study	
t *C. a. lischkei* [= *Tegula (C.) lischkei*]		m18	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
t *C. nigricolor*	m36	m18	[3]	Shirahama, Wakayama, JAPAN	present study	

t *Omphalius nigerrima*	m36	m18	[3] Type; 14M, 4SM FN=72 TCL=89.37 CL; 1.75–3.01	Shirahama, Wakayama, JAPAN	present study	
t *O. nigerrima* [=*T. (O.) nigerrima*]		m18	[1, 2]	Shimonoseki, JAPAN	Nishikawa (1962)	
t *O. pfeifferi carpenteri* [=*T. (O.) p. carpenteri*]		m18	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
t *O. rusticus* [=*T. (O.) rustica*]	m36	m18	[1, 2]	Shimonoseki, JAPAN	Nishikawa (1962)	
STOMATELLIDAE						
Broderipia iridescens	m36	m18	[3] Type; 14M, 2SM, 2ST FN=68 TCL=95.75 CL; 1.63–3.97	Shirahama, Wakayama, JAPAN	present study	
TURBINIDAE						
Astralium haematragum	m18		[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
Batillus cornutus [=*Turbo (B.) cornutus*]	m18		[1, 2]	Shimonoseki, JAPAN	Nishikawa (1962)	1
Lunella coronata coreensis	m36	m18	[3] Type; 13M, 3SM, 2ST FN=68 TCL=83.53 CL; 1.49–3.58	Shirahama, Wakayama, JAPAN	present study	
L. c. coreensis		m18	[1]	Shimonoseki, JAPAN	Nishikawa (1962)	
L. c. coreensis	m36		[2] Type; 13M, 3SM, 2ST FN=68* TCL=81.71	Mukaishima Is., Hiroshima, JAPAN	Komatsu (1984)	2

* subfamily names of Trochidae indicated by an abbreviation of small letter before the species name: c, Cantharinae; g, Gibbulinae; ma, Margaritinae; mo, Monodontinae; and t, Tegulinae.

Notes: 1. No difference in chromosome number observed between non-spine form and spine form.
2. The author calculated the value of FN as 72.

See text for further explanations.

4. Trochidae, Turbinidae and Stomatellidae (Trochacea)

Trochaceans are marine prosobranchs which have undergone wide adaptive radiation. The seven families are predominant in comparatively shallow warm seas, and nearly 1000 species are known today. Especially members of the families Trochidae and Turbinidae are noteworthy in diversity and abundance.

In the Trochidae, chromosomal information from 14 species, mostly from Japan, has so far been reported (Table 16), and except for *Grauata lyrata* all have the same chromosome number of N=18. There is reliable chromosome information on only three turbinids from Japan, and all of them are reported to have the same chromosome number of N=18.

Nishikawa (1962) noted: "Chromosomally the eight species of Trochidae studied here except *Stomatella* [=*Granata*] *lyrata*, and three species of the Turbinidae are alike in chromosome number, the arrangement of the bivalents in the nuclear plate, and the ratio of large size bivalents and small ones. On the light of above findings it is probable that the above two families are in close affinity." In the present study four species of Trochidae are newly investigated and revealed also to have the chromosome number of N=18. Chromosome morphologies have so far been studied in only a few species of the two families, and in all of them the karyotype is quite similar (see Tables 7 & 8; and Komatsu, 1984). The chromosomes have a small size-range in length and the total of the diploid chromosome lengths varies from 80.46 to 89.37 μm. The majority of the complements are M and the rest are SM/ST chromosomes; T chromosomes have not been reported in their karyotypes.

These karyological findings are in contrast with a clear divergence in their characters, such as shell structure, shell shape and opecular structure. However, the anatomical similarity of trochacean families is a remarkable fact, considering the high diversity of their skeletal characters (McLean, 1981). Therefore conservation in karyotype of major trochacean families seems to reflect their anatomical affinity. This is likely supported by the present study on a stomatellid, *Broderipia iridescens*. Though there is a slight difference between the karyotype of this species and the other trochaceans in chromosome size, its chromosome number (N=18) and its type of chromosomes (FN=68) are quite identical to those of other trochaceans.

An exception to these basic karyotypic similarities has been encountered in *Granata lyrata**. Nishikawa (1962) counted N=21 and I observed N=20 in haploid

*The result reported by Nishikawa (1962) on this species has been treated unreasonably. Patterson's list (1967) ommited it though the other data by Nishikawa were recorded. However, it was then brought back on her later list (1969). Vitturi et al. (1982) presented Nishikawa's count of the chromosome number of *G. lyrata* but mistook this for a mesogastropod Littorinidae species.

Granata lyrata used to be known as *Stomatella lyrata* and was assigned to the family Stomatellidae, sometimes placed as a subfamily Stomatellinae of the Trochidae. Studying southern Australian molluscs, Cotton (1957) introduced the new genus *Granata* for *Stomatella imbricata*. He placed this genus in the family Trochidae and further in subfamily Margaritinae, "though this genus and other closely related genera, *Herpetpoma*, *Euchelus* and *Danilia*, were somewhat different from the typical Margarites group." The name of a Japanese species, *Stomatella lyrata*, was changed into *Granata lyrata* (Kuroda et al., 1971), and at the same time they put this genus in the subfamily Monodontinae because of the radular resemblance (Habe, pers. comm.).

of *G. lyrata* in the present study, thus this species may have at least not less than the chromosome number of other trochids, N=18. Nishikawa noted, "The ancestral type of Archaeogastropoda might have been evolved from a species with a considerable higher number of chromosomes, and *Stomatella* [=*Granata*] *lyrata* has a larger chromosome number because it is placed in the subfamily Margaritinae, and may be primitive rather than the other trochus in the course of the evolutionary development." But if this species belongs not to the Margaritinae but to the Monodontinae as assorted by Kuroda et al. (1971), then what Nishikawa expected should be reversed; for the Monodontinae are to some extent intermediate between Gibbulinae and Calliostomatinae (Fretter & Graham, 1977) and those groups are thought to be advanced subfamilies in Trochidae.

As the trochaceans show chromosomal conservation in number and morphology, the distinct chromosome number of *G. lyrata* is worth noting. Further cytological study is necessary to determine if chromosome numbers different from N=18 are common or are an isolated occurrence in *G. lyrata*. This species, or the genus *Granata*, should be treated with considerable details to determine its taxonomic status in Trochacea.

Review of Karyotypes

Early works in archaeogastropod cytogenetics generally aimed at the establishment of chromosome counts often using meiotic preparations, and the detailed cytogenetic analysis on the snails is a field which has only recently been developed in a serious manner. Accordingly, Archaeogastropoda has never been characterized karyologically. In the present chapter I will review and remark on some karyological characters, i.e. chromosome number, morphology, size and sex chromosomes.

1. Chromosome number

In Archaeogastropoda, 76 species of nine families have been karyologically investigated. Table 17 shows the distribution of the haploid chromosome numbers (N) in the families of this order. The number of haploid chromosomes ranges 9 to 21 and shows trimodal distribution: the peak at 10 (and 9) being represented by the Patellacea, that at 12 by the Neritidae, and that at 18, the most frequent chromosome number in this order. There seems to be two distinctive groups of the smaller chromosome numbers of N=9, 10 or 12 and of the higher number of N=18; the members of the chromosome numbers of N=13–17 are shared by the Haliotidae and the Fissurellidae. There is a possibility that the more species of these two families are investigated, the more the members of N=13–17 may increase.

Table 17 shows that nearly all the species of each group have the same chromosome number in most groups, while in Haliotidae and Fissurellidae there is some range of variation.

2. Chromosome morphology

The morphology of mitotic metaphase chromosomes has been reported for

Table 17. Haploid chromosome numbers in Archaeogastropoda.

Family	Total of examined species	Reported species number(1)/Haploid chromosome number												
		N=9	10	11	12	13	14	15	16	17	18	19	20	21
Acmaeidae	[14]	—	14	—	—	—	—	—	—	—	—	—	—	—
Patellidae	[5]	5	—	—	—	—	—	—	—	—	—	—	—	—
Neritidae(2)	[23]	1	1	1	22	—	1	—	—	—	—	—	—	—
Haliotidae	[7]	—	—	—	—	—	2	—	2	—	4	—	—	—
Fissurellidae	[6]	—	—	—	—	1	1	1	3	—	—	—	—	—
Trochidae(2)	[14]	—	—	—	—	—	—	—	—	—	13	—	1	1
Turbinidae	[3]	—	—	—	—	—	—	—	—	—	3	—	—	—
Stomatellidae	[1]	—	—	—	—	—	—	—	—	—	1	—	—	—
Helicinidae	[3]	—	—	—	—	—	—	—	—	—	3	—	—	—
Total	[76]	6	15	1	22	1	4	1	5	0	24	0	1	1

The numbers of species reported for each chromosome number are presented.
(1): species of revised or apparently miscounted chromosome number are not listed.
(2): including one species reported to have various chromosome numbers.

Table 18. Distribution of the ratios of fundamental number to diploid chromosome number (RFD).

Family	Total of examined species	RFD=100–109	110–119	120–129	130–139	140–149	150–159	160–169	170–179	180–189	190–199	200
Acmaeidae*	[13]	—	—	—	—	—	—	3	3	4	2	1
Patellidae*	[5]	—	—	—	—	—	—	1	—	4	—	—
Neritidae**	[10]	—	—	—	—	—	1	1	—	3	1	4
Haliotidae	[4]	—	—	—	—	—	—	—	—	3	1	—
Fissurellidae	[5]	—	—	—	—	—	—	1	—	1	1	2
Trochidae	[4]	—	—	—	—	—	—	—	—	3	—	1
Turbinidae	[1]	—	—	—	—	—	—	—	—	1	—	—
Stomatellidae	[1]	—	—	—	—	—	—	—	—	1	—	—
Total	[43]	0	0	0	0	0	1	6	3	20	5	8

RFD=FN÷2N×100. The value of RFD=200 indicates that all chromosome complements consists of meta- and/or submetacentrics, while RFD=100 indicates that all consist of telo- and/or subtelocentrics. *: the distinct smallest No. 10 (in Acmaeidae) and No. 9 (in Patellidae) pair of chromosomes, regardless of morphology, calculated to have a fundamental number of 2. **: including one species with two different values of the RFD.

43 species of eight families. Table 18 shows the distribution of the RFD values: the ratio of the fundamental number (FN) to the diploid chromosome number (2N), i.e. FN/2N×100, in Archaeogastropoda. The percentage of telo- and subtelocentrics in the karyotype is very low and over half of the chromosome complements are meta- and submetacentrics in all species. The lowest value of RFD is found in a brackish water neritid, *Clithon oualaniensis*; five out of 11 of the autosome pairs are T chromosomes (Komatsu, 1983). However, there is no family characterized by karyotype consisting entirely of M/SM chromosomes as is found in some pelecypod families (Nakamura, 1985c). As is indicated by the mode of RFD values, 180–

189 (average 184), 10–20 per cent of the chromosome complements of the archaeogastropods were usually ST/T chromosomes.

3. Chromosome size

As all of the organism's genomic DNA resides in its chromosomes, chromosome size and number will reflect the size of the whole genome. Despite this, less attention has been paid to the length of the mitotic metaphase chromosomes (CL) or the total length (TCL) than to the chromosome numbers of molluscs.

To date, according to the published data and measurements by the present author based upon the published photographs, the CL in gametocytes falls in the range of 0.67–6.29 μm and the majority are between 1–4 μm, which corresponds closely with the data on that of the bivalves (Nakamura, 1985c). Such extremely small chromosomes as the supernumerary, accessory or so-called B-chromosomes in many kinds of animals and plants have not been found in Archaeogastropoda. Even the unique smallest chromosomes in Patellacea are not so small as the michrochromosomes in Aves. Very large chromosomes, larger than 10 μm, found for example in some mammals, have not been recorded at the mitotic metaphase in Archaeogastropoda, either.

Chromosomes in the early cleavage of eggs are much larger than those of the spermatogonia as was pointed out on the pelecypods, genus *Perma* (Ahmed, 1974). Chapin & Roberts (1980) reported a chromosome length in the diploid about 2–10 μm from early embryos in some western American acmaeid limpets, which is about twice as large as what I observed in several Japanese limpets (Nakamura, 1982a, b; and the present study) and Vitturi et al. (1982) in an Italian species.

TCL is known for 34 species, which ranges from 36 to 113 μm with a mean of 61 μm. Fig. 35 shows the relationships between 2N and TCL in Archaeogastropoda. With regard to the 27 species reported by myself, the two factors are positively correlated: the value r (correlation coefficient) is 0.916, which for df (degree of freedom) =26 is significant at the 0.01 level (the data of *Nerita striata* is excluded because of the apparent overcondensation of its measured chromosomes). Including all the data, presented by other investigators or measured by me on the photographs published by the original authors and the data on *N. striata* together, positive correlation is again indicated ($r=0.881$, $df=34$ and $p<0.01$; intraspecifically polymorphic *Neritina violacea* 'races' were treated separately). Therefore TCL seems to increase as 2N increases. TCL of the Trochacea are nearly twice as much as that of the Patellacea.

4. Sex chromosome

Many workers postulated the existence of a chromosomal sex-determining mechanism in Neritidae and found a heterochromatic chromosome in male meiosis and different diploid chromosome numbers in males and females. After a detailed study on both meiotic and mitotic chromosomes of some neritid species, Nakamura (1985a) concluded that they have one, non-paired, sex chromosome in the male diploid and the XX–XO type of sex determination mechanism as suggested

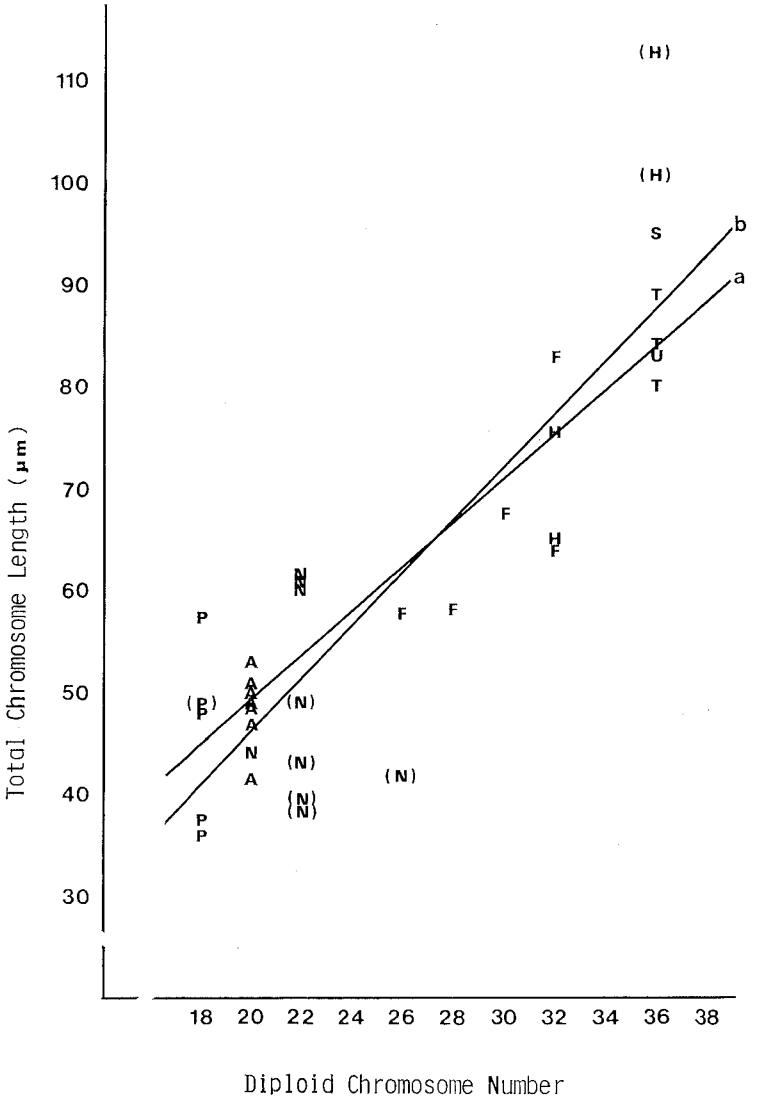

Fig. 35. Relationship between the total autosome length (TCL) and the diploid autosome number (2N). Capital letters indicating the family name to which each species belongs, A, Acmaeidae; F, Fissurellidae; H, Haliotidae; N, Neritidae; P, Patellidae; S, Stomatellidae; T, Trochidae; and U, Turbinidae. Twenty-seven species studied by the present author, indicated by letters without parentheses, show correlation between TCL and 2N (r=0.916 at the 0.01 significant level). The equation line (a) is Y=2.186X+5.394. Including all the data on 34 species, intraspecifically polymorphic *Neritina violacea* 'races' represented separately, TCL and 2N show also correlation (r=0.881 at the 0.01 significant level). The equation line (b) is Y= 2.569X−4.812.

by Natarajan (1969), and Komatsu & Inaba (1982). The sex chromosome is allocyclic in male meiosis. It is non-homologous, and thus does not pair during male meiosis, being observed as a heterochromatic univalent.

This sort of sex-determining mechanism has never been reported in the other

members of the Archaeogastropoda. An odd number of chromosomes in the diploid has been found only in Neritidae, and therefore male XO type sex determination cannot exist in the members other than the neritids.

The generalized model of sex chromosome evolution assumes that sex chromosomes have evolved from undifferentiated autosomes that carried the sex-determining genes (for example, reviewed by Bull, 1983). After a gradual accumulation of difference, they become extremely heteromorphic: the XX–XY system. The XX–XO systems are thought generally to be derived from XX–XY system by losing the Y chromosomes. I have studies so far the chromosomes of 30 species besides the Neritidae, and have never observed heterochromatic chromosomes in male meiosis or heteromorphic pairs in male mitosis. Therefore I conclude that extreme sex chromosome differences have not evolved in the groups of Archaeogastropoda except for the Neritidae. However, there is a possibility that slightly differentiated sex chromosomes without heteropycnosis in meiosis may occur in Archaeogastropoda: the application of other techniques such as banding pattern stains can be necessary to recognize them. As female chromosomes have scarcely been investigated, heteromorphic pairs in females might be found, of course, in the future, although in most groups of bisexual animals it is the male sex which is heterogametic (White, 1973).

Previously I implied a relationship between the chromosomal sex determination mechanism and the reproductive characteristic of internal fertilization with a cephalic penis (Nakamura, 1985a). If this is true, other molluscs which have developed internal fertilization may be found to have sex chromosomes. Two more groups are known to have a well-developed penis permitting internal fertilization in Archaeogastropoda; terrestrial helicinids, with a pallial gonoduct and internal fertilization by spermatophores quite possibly have such a chromosomal sex-determining system. In the acmaeid subfamily Tecturinae, Golikov & Kussakin (1972) showed ovoviviparity in some species. These species were reported to have a penis and internal fertilization system. Accordingly, it is possible that future cytological investigations on them will show chromosomal differences between the sexes.

Phylogeny and Chromosomes

With the increasing knowledge on molluscan chromosomes, the general reviews on the karyological attributes of molluscs have been presented mostly by Burch and Patterson since the later half of the 1960's. The chromosome studies of Euthyneura (Opisthobranchia and Pulmonata) were surveyed by Burch (1965), and those of Streptoneura (Prosobranchia) by Patterson (1967). From the biogeographical viewpoint, Burch (1967) compared the results of karyological studies on the Pacific Gastropoda. Then Patterson (1969) made a general review of all molluscan chromosome studies reported after 1930. The following two hypotheses can be deduced from these reviews as a karyological chief tendency among the molluscs:

1. The chromosome numbers are constant to a great extent at each of the various taxonomical levels, though some of the animals are geographically very widely separated (Burch, 1961 & 1967; Patterson, 1967; and others).

2. Increased specialization with evolutionary advancement has been accompanied by a tendency towards an increase in chromosome number (Burch, 1965; Butot, 1967; and others).

Minichev (1974) criticized these hypotheses, especially the second one, as they have often been treated as if they were general 'principles' in molluscan systematics and phylogeny, and had been applied blindly to the solution of taxonomic and phylogenetic problems. Recently the hypotheses opposite to the second one has proposed by some workers (e.g., Minichev, 1974; Vitturi et al., 1982). The idea of Vitturi et al. came originally from studies on invertebrate cytology other than molluscs (Colombera & Lazzaretto-Colombera, 1978). In this section I discuss whether these hypotheses or 'principles' are applicable also to Archaeogastropoda, and examine the modes of karyotypic evolution in this order.

1. Chromosome conservation

A general conservation with regard to chromosome characteristics is evident in three major archaeogastropod groups: Patellacea (Acmaeidae and Patellidae), Neritacea (Neritidae), and Trochacea (Trochidae, Turbinidae and Stomatellidae). These archaeogastropods have certain characteristic karyotypes including chromosome number, size and morphology, and only a few species having karyotypes that deviate from the 'basic' karyotype for their particular group. It is not unusual for several, or all members of a higher taxa to display a similar chromosome morphology and the same chromosome number; this is true not only of the Mollusca (Patterson, 1969; Nakamura, 1985c) but of other phyla as well (reviewed by White, 1973). This fact is in clear contrast with other characters in the archaeogastropods, such as anatomical, morphological and ecological ones. Thus it seems that the rate of karyological evolution, as indicated by the scarce numerical and structural changes, is very low in the major groups of Archaeogastropoda when compared to other phenetypic characters. However, considering the fact that ST/T chromosomes show a low rate in archaeogastropod karyotypes (see Table 18), a striking conservation in chromosome number is not cytotaxonomically strange in molluscan field; for I previously found in Bivalvia that the variability of the chromosome number in a certain taxa and the ratio of ST/T chromosomes in the karyotype of each species are positively correlated (Nakamura, 1985c). In major groups of the Archaeogastropoda, the chromosome pattern may have persisted substantially unaltered through long evolutionary stages.

Exceptions to this mode of karyological conservation in Archaeogastropoda are encountered in Haliotidae and Fissurellidae. When a variation in chromosome number was observed within the family or other taxa, it was regarded as representing an unnatural group (for example, Butot & Kiauta, 1969; Lo & Chang, 1975) or as caused by the technical faults to be ignored (for example, Colombera &

Tagliaferri, 1983) because chromosome conservation has too often been emphasized in several molluscan groups. In this way the importance and biological meanings of chromosome diversification have been scarcely discussed in the field of molluscan cytotaxonomy. This appears to me an arbitrary treatment of the facts, relying upon the proposition of chromosomal conservatism, and thus I am provoked not to neglect but to appreciate the chromosomal variability found in Haliotidae and Fissurellidae as a prime cytotaxonomical attribute of these groups and reflecting the contributions of karyotypical change in their origination.

On the assumption that karyotypical alternation is concerned with the origination of the Haliotidae and the Fissurellidae, the following suppositions can be deduced based on the achievements of modern cytogenetics: (1) Their lineages evolved promptly with drastic alternation of genetic background caused by chromosomal change, which has been called 'catastrophic selection' and 'saltational chromosome speciation' on plants by Lewis (1962). This mode of speciation is thought to be common in animals as well (White, 1978). (2) Their origination are rather late in the Archaeogastropoda according to the 'canalization model' of chromosomal evolution (Bickham & Baker, 1979); for the age of a lineage can be negatively correlated with karyotypic variability under this model. Bickham (1984) noted, "the canalization model of chromosomal evolution hypothesizes that extensive karyotypic rearrangement occurs during the initial radiation" of a group, and "the lineage evolved through a period of intermediate rate of change and into a period of karyotypic stability."

The Haliotidae and the Fissurellidae have a limpet form which is thought to have been derived from coiled predecessors (McLean, 1981). This may support the above first supposition of prompt origination deduced karyologically; for according to McLean (1984), "the origin of limpet groups [except for docoglossate patellaceans] can be regarded as paedomorphic in the sense that limpets are sexually mature post larval gastropods because they remain uncoiled.... A paedomorphic transition acts upon the developmental process and has to be a rapid event." Then it is naturally assumed that species with a limpet form represented in many diverse families of gastropods can be found to have chromosomal variability or to differ in karyotype from their coiled predecessors. So far, however, karyological information on these limpet-form gastropods has scarcely been available to examine this assumption, which is left for the future studies.

The age of the lineages of the Haliotidae and the Fissurellidae is one of the controversial problems of the day in the field of prosobranch systematics, but at least Golikov & Starobogatov (1975) noted that these are high ranking families in Scutibranchia [=Bellerophontina and Zygobranchia], and the Haliotidae is phylogenetically young, and the Fissurellidae is the youngest group, which supports the second supposition mentioned above. In any case, therefore, it is possible to conclude that karyotypic alternation takes place at the origination of the Haliotidae and the Fissurellidae; and thus the karyotypes of these families unlikely reflect their ancestral karyotype itself.

Another example of the karyotypic variability is found in non-marine neritids. The Neritacea is thought to be a very old group and to show an independent line of evolution in Prosobranchia (Cox, 1960; Salvini-Plawen, 1980). The marine members in this group show extreme karyotypic identity. On the other hand members living in brackish/fresh-water areas reveal karyotypic divergence. The 'canalization model' can be a good explanation of the karyotypic mode in neritids; according to this model, lineages experience rapid chromosome evolution when they first break into a new broad adaptive zone and thus show high divergence in karyotype (Bickham, 1984). Therefore, not only the non-marine neritids can be surely regarded as evolved from the marine members, but also evolution from their marine ancestry is possibly rather a recent event.

2. Chromosome evolution

It is one of the major subjects in cytotaxonomy to examine the polarity in karyotype change (Imai & Crozier, 1980). Burch (1965) was of the opinion that chromosome numbers do not necessarily reflect primitive or advanced conditions among Mollusca, but he thought they may have a tendency to increase with increasing specialization and evolutionary development (see Patterson & Burch, 1978): an idea named 'increase hypothesis' tentatively. A different view was expressed by Butot & Kiauta (1969) or Vitturi et al. (1982), who suggested that primitive members of molluscan groups have large numbers of chromosomes, the number being reduced in more specialized form: 'decrease hypothesis'. These hypotheses were both deduced from adopting the karyological data (usually chromosome numbers) to the phylogenetic relationships of some molluscan groups which were proposed on the basis of morphological/anatomical studies. Naturally, depending on the phylogeny the same karyological condition can be interpreted in different ways. For instance, Vitturi et al. (1982) mentioned, "we find the lowest number of chromosomes in the families Acmaeidae and Patellidae within the order Archaeogastropoda, in the order Nudibranchia within the subclass Opisthobranchia..., all of which are considered to be specialized groups." Acmaeidae and Patellidae were thought to be a specialized and relatively recent derivation, probably following the formerly accepted phylogeny of Fretter & Graham (1962), who assorted these docoglossate groups as derived from a zygobranch ancestor. On the other hand Golikov & Starobogatov (1975) declared that the docoglossate patellaceans (i.e., Acmaeidae, Patellidae and Lepetidae) should be regarded as a separate phylogenetic line and are more primitive than the rest of the Gastropoda, which led them to think that a low chromosome number in this group exemplified the 'increase hypothesis'.

Apart from the attitude in the application of morphologically constructed phylogenies to the chromosome data, there can be a controversial point to these hypotheses. The 'increase hypothesis' seems to have been also deduced from some cytological aspects. Patterson (1971) noted, "Basic diploid species can tolerate duplications or additions of chromosomes more easily than loss of chromosomes,

and therefore, variation in chromosome number within a taxon probably more usually reflects addition of rather than loss of chromosomes." I do not know on what ground she could say so; decreasing chromosome number does not simply mean 'loss' of chromosome itself. For instance, Robertsonian translocation can result the change of the number of chromosomes without any increase or decrease of the amount of hereditary material. I can not find any cytological basis on which the 'increase hypothesis' (and probably the 'decrease hypothesis' as well) stands in the molluscan cytotaxonomical field.

The correlation between morphological and karyological evolution is difficult to understand (Bickham, 1984). Some phylogenetic trends can be associated with chromosome variations, but it cannot be established, at least at present, to what extent and how these two events are linked to each other. In other words, "because a group is primitive or derived morphologically does not necessarily mean the same is true of karyotype" (Bickham, 1984). In the present study, for example, though the morphological features are quite identical, their chromosome number is found to be variable in Haliotidae; and the trochaceans show chromosomal conservation in number and morphology in contrast with a wide variety of their skeletal characters. I do think that no clear polarity or directionarity in changes of chromosome number has been demonstrated in the Archaeogastropoda, whereas both a decrease and an increase may have occurred at least in the phylogeny of *Neritina violacea* 'races'.

Primarily it was noticed that the patellacean families, the Neritidae, and the trochacean families have respective karyotypes which are distinctly different from each other. There seems to be a large gap between the trochaceans and the former groups not only in chromosome number (2N) but also in total chromosome length (TCL) and fundamental number (FN). If difference in 2N were due to Robertsonian changes, FN and TCL ought to be constant or slightly variable (Matthey, 1973). In fact FN and TCL in trochaceans are not less than double of those in patellaceans. This and the sequence of numbers such as N=9 and 10 in patellaceans, and 18 and 20 (or more) in trochaceans suggest that the evolution of chromosome numbers in these groups was perhaps not a gradual alternation by duplication or elimination of existing chromosomes (aneuploidy) but rather by duplication of entire complements (polyploidy). This is also indicated by the data on the cellular DNA content (Hinegardner, 1974): DNA content of the trochaceans is over twice as much as that of the patellaceans. To be sure, the Haliotidae and the Fissurellidae show the intermediate chromosome numbers between those in Patellacea and Trochacea, but I think that their lineage evolved comparatively recently, and that their chromosome numbers are secondarily altered ones and do not connect the chromosome number of the Patellacea and that of the Trochacea as gradual intermediate between them.

Polyploidy is very widely spread among plants and has been regarded as an important mechanism of speciation in the plant kingdom, particularly among the flowering plants (see Stebbins, 1950 for a review). In contrast to plants,

polyploidy is rare phenomenon in animals. Several reasons were discussed by Muller (1925) as to why polyploidy is rarer in animals than in plants. According to him, polyploidy in animals should be confined to groups in which reproduction is hermaphroditic, parthenogenetic or colonial. Based upon the fact that hermaphroditism occurs in all classes of the Mollusca except for the most highly specialized group, Cephalopoda, Fretter & Graham (1962) asserted, "the ancestral mollusc was hermaphroditic, and although this condition may not have persisted into modern diotocardians, the tendency to produce a hermaphroditic condition is undoubtedly present." Therefore it is tempting to think that the early radiation of the archaeogastropods or the gastropods was due to polyploidy. Indeed, some patellid limpets are known to be hermaphroditic (see Fretter & Graham, 1962 for a review). On the other hand, polyploidy is incompatible with the well-established chromosomal sex-determining mechanism (Ohno, 1970), as found in the Neritidae.

Therefore, I think the different chromosome numbers found between Patellacea and Trochacea (and Helicinacea ?) is ascribable to evolutionary polyploidy occurred in the hermaphroditic ancestral molluscs, which were closely related to the patellaceans. Because of having a chromosomal sex-determining system the neritid karyotype may have derived not through polyploidy but through aneuploidy from an ancestor which had not yet undergone the evolutionary polyploidy resulting in the trochacean lineage. The karyotypes of the Haliotidae and the Fissurellidae may have independently resulted from some predecessors which had already undergone evolutionary polyploidy. The karyological studies on the Pleurotomariacea may hold keys to this hypothesis; for this primitive coiled snail group is thought to be closely related to the Haliotidae and the Fissurellidae and is the only recent superfamily not yet karyologically studied in Archaeogastropoda.

Archaeogastropoda includes diversified groups, some of which are still controversial as to their systematic status in Archaeogastropoda or even as to their inclusion among the order. As this order is thought generally to be the stem of the gastropod phylogenetic tree, derivations of systematic studies of it are to cause the re-examination of phylogenetic relationships of whole Gastropoda. The present discussions employ all the currently available, karyological evidences for Archaeogastropoda, which come from only a part of its species, actually less than three per cent of 3000 living species. Many minor families are left untouched at all and the major groups are still represented only by a small number of species. Nevertheless, the framework of karyological relationships drawn here must have some phylogenetic inference on this primitive group. With or without the application of modern banding techniques, continued study of archaeogastropod chromosomes will verify several hypotheses suggested in the present study and will aid in elusidation of many otherwise difficult questions about the relationships and evolution of Archaeogastropoda and Gastropoda as well.

Summary

1. Karyotypes of 27 species of eight archaeogastropod families were investigated

using the warm-dry chromosome method and a computer-assisted analysis system. Most of the specimens were collected from the sea shore around the Seto Marine Biological Laboratory on the southwest coast of Kii Peninsula, Honshu, Japan.

2. Twelve out of 27 species were investigated for the first time. Three of the 15 re-examined species were found to have different chromosome numbers from the results in earlier works.

3. Karyological information on the Archaeogastropoda was reviewed based on the data stored in the CISMOCH, Computerized Index System for Molluscan Chromosomes.

4. Haploid chromosome numbers (N) range from 9 to 21 in the 76 species of nine archaeogastropod families. The Patellidae (5 species, N=9), Acmaeidae (15 spp., N=10) and Neritidae (21 spp., N=12; 1 sp., N=9 & 10; and 1 sp., N =11, 12 & 14) have relatively small chromosome numbers, whereas the Trochidae (13 spp.), Turbinidae (3 spp.), Stomatellidae (1 sp.) and Helicinidae (3 spp.) have a larger chromosome number, N=18 (and one trochid *Granata lyrata,* N=20 or 21).

5. Chromosome morphologies have been investigated in 42 species. Over half of the karyotype complements are meta- and/or submetacentrics in all of them, while no families are characterized by the complements consisting of only meta- and/or submetacentrics.

6. The lengths of the chromosomes at the mitotic metaphase range mostly from 1 to 4 μm. Total diploid chromosome length has been measured for 34 species, and shows an increase as chromosome number increases.

7. Two patellacean families are characterized by one distinctly small chromosome pair, which, together with the studies on the shell structure, leads to an assumption on their phylogeny that these two families share a common ancestor and they do not have ancestor-decendent relationships.

8. The Neritidae have sex chromosomes and have the XO–XX sex-determining system. The sex chromosome shows allocycly in male meiosis and is one of the largest meta- or submetacentric chromosomes in the diploid except in *Nerita albicila*, whose sex chromosome is medium-size telocentric.

9. Robertsonian translocation and tandem fusion are postulated as a mechanism for the change in karyotype of the *Neritina violacea* 'races', an intraspecifically polymorphic neritid in chromosome number, living in brackish water areas.

10. Conservation in chromosome number and morphology is apparent in major archaeogastropod groups. Remarkable diversification in karyotype has occurred primarily in special groups having a secondary limpet-form or expanding from the sea to brackish/fresh-water areas.

11. No results have been obtained in support of the previous hypotheses on the evolutionary tendency of change in chromosome number. At least in the phylogeny of *Neritina violacea* 'races', both decrease and increase may have supposedly occurred.

12. It is hypothesized that evolutionary polyploidy occurred in a hermaphroditic ancestor at a very early stage of gastropod radiation.

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